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Cover photo: Mating in *Semeiochernes armiger* (Psudoscorpiones) collected in the Republic of Panama. Photo by Jeanne A. Zeh.

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Frame-web-choice experiments with stingless bees support the prey-attraction hypothesis for silk decorations in *Argiope savignyi*

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Abstract. There is controversy about the function of silk stabilimenta, also called silk decorations, on spiders' webs. Most of the proposed hypotheses have been tested using indirect methods. Protection against predators, advertisement for vertebrates to avoid web damage, and increasing prey attraction are the most popular hypotheses. In this study, I tested the prey attraction hypothesis on the silk decorations of the araneid *Argiope savignyi* using a trial tunnel built in the field, in which I exposed stingless bees *Tetragonisca angustula* to decorated and undecorated webs placed on wooden frames. I carried out two experiments: 1) a three-frame choice, consisting of a frame bearing a decorated web, one bearing an undecorated web and a control frame without web and spider; 2) a two-frame choice, in which the bees were exposed to only two frames consisting of "decorated web vs. control," "decorated web vs. undecorated web," and "undecorated web vs. control". In favor of the prey attraction function, I found that decorated webs intercepted more bees than webs deprived of the decoration or controls with no webs. *Argiope savignyi*'s decorations might lure prey to the web by UV-reflectance as it has been suggested for other *Argiope* species.

Keywords: Decorated, foraging, stabilimenta, undecorated

A wide range of orb-weaving spiders builds silk decorations or stabilimenta on their webs (Araneae: Araneidae, Tetragnathidae, Uloboridae; Scharff & Coddington 1997). Five functions have been suggested for these structures: 1) protection against predators, 2) advertisement to vertebrates so as to avoid web damage, 3) prey attraction, 4) stabilization of the web, and 5) a source of shade. Most work has focused on the first three hypotheses (Herberstein et al. 2000; Bruce 2006). However, after more than 100 years of research, no consensus about the functionality of decorations has yet been reached, and a variety of methods have been applied producing contradictory outcomes. In support of functions 1 and 2, decorations on the web *of Argiope aurantia* Lucas 1833 reduced predatory attacks by mud-dauber wasps and web damage by birds; simultaneously, web visibility to prey was increased and prey capture rates declined. Hence, a cost associated with decoration construction was suggested (Blackledge & Wenzel 1999). A different study with *A. aurantia*, on the other hand supported Function 3, that decorated webs attracted more prey although they were compared with undecorated webs of *A. trifasciata* (Forsskål 1775) (Tso 1998a). For *A. appensa* (Walckenaer 1842), no differences in foraging success were found between decorated and undecorated webs. In support of Function 3, Bruce *et al.* (2001) and Seah & Li (2001) found that decorated webs of *A. keyserlingi* Karsch 1878 and *A. versicolor* (Doleschall 1859) attracted more prey; however, decorations also attracted predators, in opposition to Function 1. Researchers have concluded that there is a trade-off in foraging strategies, since decorated webs are often smaller than undecorated webs (Hauber 1998).

The traditional perception that the spider web is an undetectable trap has changed drastically since the idea that web decorations might attract prey by UV reflectance was suggested (Craig & Bernard 1990). The prey-attraction hypothesis (Function 3) states that the presence of decorations increases the foraging success of the spiders. Such an outcome has been proposed for various species of the genus *Argiope*: *A. aetherea* Thorell 1881 (Elgar et al. 1996), *A. trifasciata* (Tso 1996), *A. aurantia* (Blackledge & Wenzel 1999), *A. versicolor* (Li et al. 2004; Li 2005), *A. argentata* (Fabricius 1775) (Craig & Bernard 1990; Craig et al. 2001), *A. keyserlingi* Karsch 1878 (Herberstein 2000; Bruce et al. 2001), *A. aemula* (Walckenaer 1842) (Cheng & Tso 2007); as well as for *Octonoba sybotides* (Uloboridae) (Bösenberg & Strand 1906) (Watanabe 1999), *Araneus eburnus* (Keyserling 1886) (Bruce et al. 2004), and some other species (Herberstein et al. 2000; Bruce 2006).

An important aspect to be considered when testing the prey-attraction hypothesis is the interference of web-size: decorated webs, usually smaller than undecorated ones, might attract more prey due to their decoration. Undecorated webs, however, are usually bigger and hence prey-capturing success might be increased due to the larger area. Therefore, the suggested trade-off in foraging strategies and energetic costs remains speculative. For that reason, an appropriate technique to eliminate the influence of web size, when decorated and undecorated webs are compared, has been manual removal of the decorations (Bruce et al. 2001, 2004).

I tested the prey-attraction hypothesis for the poorly studied Neotropical spider *Argiope savignyi* Levi 1968 using a new method that consisted of a trial tunnel combined with decoration removal and prey manipulation. The tunnel is placed in the field, which can mimic natural visual conditions in which spiders and preys are found. Many studies have tested the hypothesis in laboratory conditions (e.g., Y-choice experiments), which might not reproduce natural conditions. As well, the influence of web size can be eliminated while the prey capture history of the spiders, which has an essential effect on the decoration behavior (Craig et al. 2001), can be controlled. If the web decoration functions to attract prey, then I expected that decorated webs would intercept more bees than the undecorated webs and empty control frames.

METHODS

Site.—This study was carried out from 18 July to 4 August 2007 at La Selva Biological Station, Heredia, Costa Rica (10°26′N, 83°59′W), a 1550-ha reserve in the Atlantic lowlands with an annual average rainfall of 4000 mm. See Sanford et al. (1994) for more details about the station.

Animals.—*Argiope savignyi* is an aerial web weaving spider that decorates its web with zigzags of silk laid in a variety of designs that include silk discs (juveniles) or one to four arms of a cross (adults). Some webs lack decorations (Nentwig & Rogg 1988). This species is common at La Selva (Rovner 1989; Timm & Losilla 2007). I confirmed the species identity using the taxonomic key for *Argiope* by Levi (2004). No voucher specimens were collected but some collected from La Selva are available at the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (Levi 2004).

Experimental Design.—Collected individuals of *A. savignyi* were placed in a large screened cage (7 × 3 × 2 m). This cage contained herbaceous vegetation with insects such as Homoptera, Hymenoptera, Orthoptera, on which the spiders were allowed to forage. In addition, each spider was fed several stingless bees to guarantee that they were satiated, an important factor for inducing the construction of decorations (Craig et al. 2001).

A 300 × 120 × 80 cm tunnel, open at both exits, was constructed (Fig. 1). The different web treatments were set up on wooden frames at one end, and a wooden box (40 × 30 × 20 cm) with a nest of the stingless bee *Tetragonisca angustula* Latreille 1811 was placed in the other end. The frames were put on a 2 × 35 × 120 cm wooden board placed at the exit of the tunnel so that the frames were not in contact with the ground. The exit of the bee nest faced that of the tunnel for the web treatments. Bees could leave the tunnel through the exit containing the frames, which they usually did, or by the other exit. The nest was placed in the tunnel with both exits opened for 48 h before the beginning of the experiment in order to get the bees used to the tunnel and the new nest location. The reason for placing the nest entrance near the tunnel exit was to reduce the stress on the bees, which probably occurs when they are individually manipulated, for instance with CO_2 anesthesia (Bruce 2006). With the intention of comparing the two web treatments, I used spiders of similar sizes, and the control frame never contained a spider. The exit of the tunnel where the frames were placed was in front of herbaceous vegetation, and a dark green mesh placed one m from it.

I performed two experiments with *A. savignyi*: 1) A "Three-frame choice," consisting of three frames (34.5 × 45.0 cm, or 20 × 20 cm for smaller webs) placed next to each other at the same time and at the same end of the tunnel with different web treatments; one bearing a decorated web, one bearing an undecorated web, and a control without web and spider (Fig. 1) and 2) a two-frame-choice experiment in which the bees were exposed to only two frames placed at the same end of the tunnel and consisting of the following: "decorated web vs. control," "decorated web vs. undecorated web," and "undecorated web vs. control." Small frames (20 × 20 cm) did not cover the entire area of the tunnel's exit, so I covered the remaining space with cardboard sheets. For the three-frame-choice experiment, I used two spiders per replicate (n = 8, 155 bees): one for the decorated web and one for the undecorated web. For the two-frame-choice

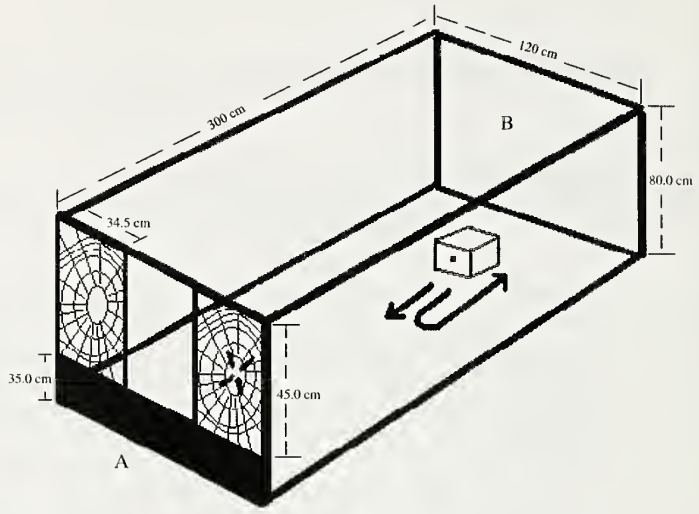

Figure 1.—Trial tunnel in which the stingless bees were exposed to the different web treatments. The walls and roof of the tunnel are removed in order to reveal the inside. Solid arrows show the two possible trajectories of bees to fly out of the tunnel from the nest (N). The exit bearing the web treatments is represented by A and the opposite exit by B.

experiments, I compared "decorated webs versus control" frames for 17 repetitions (175 bees), "undecorated webs versus control" frames for 9 repetitions (86 bees), and "decorated webs versus undecorated webs" for 10 repetitions (100 bees). The three-frame-choice experiment trials lasted approximately 5 to 15 min. Bees were allowed to return to the nest except for those that were collected in order to feed the spiders (or caught and consumed by the spider itself). Only one trial was carried out per day, which greatly reduces the possibility of avoidance learning by stingless bees (Craig 1994b). Craig (1994b) also proposed that even if bees learn to avoid decorated webs (e.g., in one location), they are unable to generalize a similar response to other decorated webs. The two- and three-frame-choice experiments were carried out in random order. The three sets within the two-frame-choice experiments were randomly assigned as well.

All decorations were either cross or linear patterns. Decorations were removed by burning the fine silk lines with heated fine-point forceps while the spider was on the web except on a few occasions when the spider was removed first. The spider was then placed back on the web after the decoration was removed. In some cases, a little damage was done to the web during burning, and in these instances, I used the forceps to produce similar damage to the decorated web.

I counted the numbers of bees either being intercepted (including bees caught by spiders) or flying through each frame, and determined the number of bees intercepted per frame. I switched the positions of the frames each time two bees had exited the tunnel or were intercepted in order to avoid any possible bias due to frame position. The frames were placed at the exit of the tunnel only when no bee was either leaving the nest or flying in the tunnel. In cases in which three or more bees accumulated in the web because the spider did not attack them, I removed the three frames and used forceps to remove the bees in order to avoid the possibility that bees caught there would deter more bees from flying into the web. I did not remove the bees if they were captured by the spider or

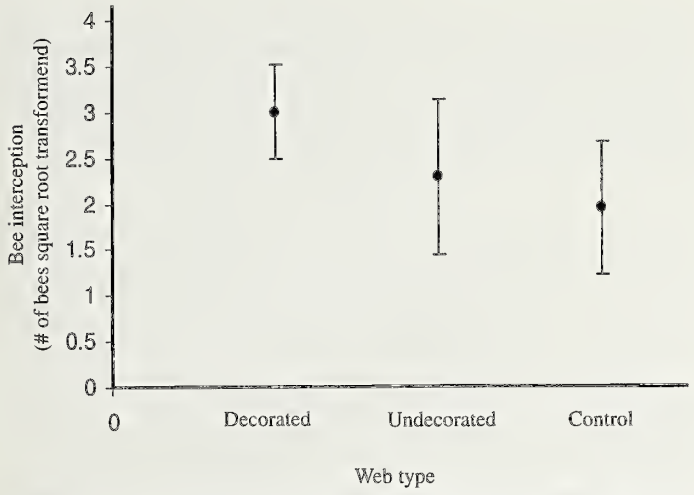

Figure 2.—Number of *Tetragonisca angustula* bees intercepted for the three-frame-choice experiment set for *Argiope argentata*. Mean ± SD.

wrapped with silk by the spider. After this, I put the frames back at the exit to continue the experiment. I used 10–30 bees per repetition, which required a new set of webs made by spiders not previously used. I did not register numbers of bees wrapped or consumed. Spiders wrapped bees few times, but they usually kept consuming the first bee that was caught. This apparently did not discourage bees from flying into the web. All the trials were carried out at 09:30–12:00 and 13:00–15:00 h, when the light conditions were relatively constant.

The counts from the three- and the two-frame-choice experiments were square root transformed. The transformed data from the three-frame choices was tested for normality and analyzed using a single factor ANOVA. Finally, the transformed data from the two-frame-choice experiments were analyzed with a *t*-test for paired samples. Effects were accepted as statistically significant if $P \leq 0.05$, and all analyses were done using STATISTICA 7.0 (StatSoft 2001).

RESULTS

Bees were intercepted by decorated webs significantly more often than by the undecorated webs or the control frames (48, 30 and 22% of the bees respectively, $F_{2, 21} = 4.65$, $P = 0.02$, Fig. 2). I did not find significant differences between undecorated webs and the control frames (Tukey HSD test, $P = 0.362$). In the "decorated webs versus control" experiment, 64% of the bees chose the frames bearing decorated webs ($t = 2.84$, $df = 16$, $P = 0.006$, Table 1). Decorated webs also intercepted more bees (58%) than undecorated webs (42%), $t = 1.91$, $df = 9$, $P = 0.044$).

DISCUSSION

The prey attraction function of silk decorations for *A. savignyi* is supported by both the two- and the three-frame-choice experiments (Fig. 2, Table 1). Decorated webs intercepted significantly more bees than those webs from which the decoration was removed. Webs deprived of decorations showed no significant differences from the control frame that lacked either web or spider. The results from these experiments are also reinforced by the fact that bees did not show an avoidance-learning process which would have decreased the interception of the decorated web (Craig 1994a). The literature covering this hypothesis is controversial; many studies have revealed that decorated webs intercept more prey than undecorated webs (Craig & Bernard 1990; Elgar et al. 1996; Tso 1996, 1998a, 1998b; Watanabe 1999; Herberstein 2000; Bruce et al. 2001; Craig et al. 2001; Li et al. 2004; Li 2005; Bruce & Herberstein 2005; Cheng & Tso 2007) but some studies found no evidence in favor of the hypothesis (Blackledge 1998; Blackledge & Wenzel 1999; Hoese et al. 2006; Jaffé et al. 2006; Bush et al. 2008; Eberhard 2008; Gawryszewski & Motta 2008). This hypothesis has been previously supported for one of the closest relatives of *A. savignyi*, *A. argentata* by Craig (1991) and Craig et al. (2001), but no manipulative experiments (e.g., decoration removal) were performed. Craig et al. (2001) correlated the increase in decoration frequency with the increase in the number of stingless bees. Craig (1991) also calculated an index of predator-prey encounter rates based on the damage found on the web. Such damage is not necessarily caused by prey, however. She also assumed that the prey damage or destroy part of the web, even when they are not captured. I saw several cases in which a bee was intercepted in the web and later escaped without damaging the web.

There is one result from the set "undecorated web versus control" that clearly merits further study. It could be anticipated that the undecorated webs (bearing a spider) would intercept more bees than the control, considering the UV reflective properties of the spider's dorsal surface that is thought also to play an important role for attracting prey (Craig & Ebert 1994; Cheng & Tso 2007; Bush et al. 2008). This was not observed but partially supported by the three-choice-frame experiments; 30% and 22% bees intercepted undecorated and control frames, respectively. Yet the bright coloration of *Argiope* spiders may have no relation as a prey attraction function, serving more as camouflage for the spiders in *A. bruennichi* (Václav & Prokop 2006) and *A. keyserlingi* (Hoese et al. 2006). The functional significance of body coloration of *Argiope* spiders remains unresolved.

One of the advantages of the method in this study was that stress on prey was reduced, since the experiments were performed in the field. The prey attraction hypothesis can be

Table 1.—Statistical summary and preferences for the two-frame-choice experiments set for *Argiope argentata*. dec: decorated webs; undec: undecorated webs; and control.

Treatment	*t*	*df*	*P*	Total number of bees	% of bees intercepted		
					dec	control	undec
dec vs control	2.84	16	0.006	175	64	36	----
undec vs control	0.22	8	0.42	86	----	49	51
dec vs undec	1.91	9	0.04	100	58	----	42

directly tested in a number of ways, all of which have their advantages and disadvantages. The field correlation technique involves correlating the presence of web decorations with prey capture rates, but this method has produced contradictory results for different species (Hauber 1998; Tso 1998b; Herberstein 2000; Bruce et al. 2001; Craig et al. 2001; Bruce et al. 2004). A negative aspect of field correlations is that the prey capture history of the spiders is unknown, and satiated individuals can construct more decorations (Blackledge 1998; Tso 1999; Seah & Li 2002). Consequently, decorated webs may just be in sites where prey are abundant.

Another method is the Y-choice experiment, which has been used in laboratory experiments to show that flies are attracted to decorations (Craig & Bernard 1990; Watanabe 1999; Bruce et al. 2001; Li et al. 2004). These studies have been carried out in laboratory conditions using artificial lights. Moreover, decorated and undecorated webs have been contrasted without the presence of the spider on the web, which might reduce the similarity to a natural prey-spider encounter. The third technique is the experimental manipulation of webs by decoration removal to compare decorated and undecorated webs. This allows investigating the effects of these structures on prey capture and predator response without the cause and effect problem as in the field correlation method (see Bruce 2006). Some studies in which decoration removal was used in the field found opposite results for the prey-attraction function. Blackledge & Wenzel (1999) suggested that the decoration in *Argiope aurantia* reduced foraging success, but Tso (1998a) found that the decoration in fact increased it. The possible reason of this difference is that the former study did not control for web size and the latter one used webs of similar size, as I did.

Even though the prey-attraction function is supported by these experiments, other hypotheses, such as advertisement to avoid web damage by vertebrates (Blackledge & Wenzel 1999) and the anti-predator function (Bruce et al. 2001; Schoener & Spiller 1992), are not necessarily discardable. Scharff & Coddington (1997), in their phylogenetic analysis of the family Araneidae, proposed that web decorations evolved nine times independently in the 15 genera in which they are known to occur. Although they reasoned that the widespread convergent evolution of this trait only in diurnal species suggests a search for a common cause, it might be possible to find a wide range of function across the different groups of spiders that evolved this trait. Is a multifunction role possible for *Argiope's* web decorations? For instance, *Argiope trifasciata*'s decorations increase foraging success (Tso 1996, 1998a) and also provide protection against predators (Blackledge & Wenzel 2001). For *A. aurantia*, the prey attraction, predator avoidance, and web advertisement functions have found support (Tso 1998a; Blackledge & Wenzel 1999). However, some studies that addressed a multifunction role only found evidence for one function (e.g., Blackledge & Wenzel 1999; Bruce et al. 2001). Bruce & Herberstein (2005) found differences in the decorating behavior of three Australian *Argiope* species that were apparently related to the pattern of decoration that each species built. These dissimilarities suggest that those decoration patterns perform different functions, although with different costs and benefits associated. The other two important visual functions suggested for decorations, the anti-predator and the web advertisement hypotheses can be tested using a similar approach, employing manual decoration removal in a more natural visual condition similar to this study. In this way, the different web types and their spiders can be exposed to either predator or vertebrates (e.g., birds) in order to quantify their behavioral responses.

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Conflict or cooperation in the courtship display of the white widow spider, *Latrodectus pallidus*

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Abstract. We used experimental manipulations to test adaptive explanations for the courtship display of the male widow spider, *Latrodectus pallidus* O. Pickard-Cambridge 1872. Two hypotheses have been suggested to explain a long and complex male display: a) Cooperation of males and females in the effort to physically stimulate the female. As the time of male arrival is not predictable, females may delay sexual readiness until the appearance of a courting male. b) Conflict between males and females regarding the display cost. Females impose on the males an energetically costly display that may last several hours as a test of their quality. To test both hypotheses, we manipulated the previous experience of either the male or the female. We presented naive or experienced males (males that had courted and were accepted by females but were prevented from copulating) to females that were either naive or experienced (had been courted by a male but prevented from copulating). We also presented naive males to mated females. Following the stimulation hypothesis, courted females were presumed to have been stimulated to mate and thus were expected to accept non-courting males as mates. Both naive and mated females, however, were expected to await male stimulation before allowing copulation. In contrast, the conflict of interest hypothesis predicts that the female tests each male for quality indicators and therefore a non-courting male should not be accepted as a mate. Mated females, however, should apply a less stringent test to courting males. Our results show that 1) naive females prevented males that did not perform a full courtship display from entering the nest and mounting; 2) naive males courted virgin females with the full display, independent of the female previous courting history; and 3) naive males shortened their courtship when presented with mated females. The results are consistent with the conflict of interest hypothesis.

Keywords: Sexual stimulation, male quality, sexual conflict, Theridiidae

Elaborate, conspicuous and time-consuming precopulatory displays are known in many animal taxa, including insects (Thornhill 1976; Svensson et al. 1990), fishes (Milinski & Bakker 1990), birds (Borgia 1995), and mammals (Behr & von Helversen 2004), with several hypotheses suggested for their function. These hypotheses, which are not necessarily mutually exclusive, postulate either a mutual interest of both male and female in courtship or a conflict between the two. Mutual interest may occur if the courtship enhances reproductive isolation (Mayr 1963; Dobzhansky 1970) by providing cues for species recognition (Ryan 1985; Andersson 1994) or if a long courtship is necessary to stimulate the female into mate (see reviews in Platnick 1971; Robinson 1982). A conflict may arise between males wanting to increase their fitness by mating as often and with as many females as possible, and females who increase their fitness by choosing the best male available (Trivers 1972; Parker 1979; Eberhard 1996). Conflict occurs when females choose males based on their courtship display, and subsequent escalation of the display imposes an increasing cost on the males, which reduces the males' fitness and the potential to mate with additional females (Andersson 1994; Eberhard 1996). Nevertheless, the male and female usually share an interest in mating, particularly when the chance of encountering mates is small (Segoli et al. 2006).

Some species of spiders have elaborate and costly precopulatory displays, including cutting the female's web, vibrating on the female lines, and drumming vigorously on the substrate (e.g., Robinson & Robinson 1980; Suter & Renkes 1984; Forster 1995; Parri et al. 1997). Understanding the role of males and females in shaping the courtship display in spiders is challenging in light of the cannibalistic behavior of females in many of these species (Elgar & Schneider 2004), since cues allowing for species recognition to avoid predation may be similar to those of mate assessment (Robinson & Robinson 1980; Andrade 1996; Schneider & Lubin 1998; Herberstein et al. 2002).

In order to test these two general explanations for the male courtship display, we investigated the courtship behavior of the white widow spider, *Latrodectus pallidus* O. Pickard-Cambridge 1872 (Theridiidae), inhabiting the Negev Desert, Israel, which belongs to a genus known for its sexually cannibalistic behavior (Ross & Smith 1979; Breene & Sweet 1985; Forster 1995; Andrade 1996; Segoli et al. 2006). Females of *L. pallidus* are large, sedentary predators, while adult males are less than a third of the female's size and, as in other *Latrodectus* species, actively search for females (Segoli et al. 2006). The female's web consists of a nest located in a shrub and connected by strong threads to a capture web consisting of a loosely woven platform and thin, prey-capture threads stretching from the platform to the ground (Lubin et al. 1991). In a similar species found in the same habitat, *L. revivensis* Shulov 1948, the male is attracted to the female's web by means of a female sex pheromone associated with the web silk (Anava & Lubin 1993). On the web, males engage in a vibratory display while cutting and removing sections of the capture web before approaching the female's nest and engaging in tactile courtship (Segoli et al. 2006, 2008). The courtship behavior has been described in several species of

Latrodectus (Kaston 1970; Ross & Smith 1979; Lubin & Anava 1993; Forster 1995), but not in *L. pallidus*.

To determine what factors shape the courtship display in *L. pallidus*, we tested two hypotheses concerning the function of the display and the context in which it is given: 1) cooperation between the partners aimed at stimulating the female for mating, and 2) a signal of male quality used by the female when male and female interests over mating potentially conflict.Under the first hypothesis, sedentary, virgin females that wait in their nest for the arrival of conspecific males must be physiologically stimulated before they are ready to mate (Robinson & Robinson 1980; Suter & Renkes 1984). The courtship display may provide the trigger that sexually primes the female (Platnick 1971; Robinson 1982; Anava & Lubin 1993). This hypothesis predicts that the male courts the female until she signals her willingness to mate, and only upon receiving this message will the male enter the female's nest and attempt to copulate. According to the alternative hypothesis of conflict over mating, information provided by the male during his display enables the female to arrive at a decision whether to allow him to continue courting and later to copulate (e.g., Bukowski & Christenson 1997). This hypothesis predicts that a choosy virgin female should demand a lengthy courtship, while the male will attempt to reduce the effort he puts into courtship in order to lower the energetic cost of the display. The courtship display of many spider species consists of lengthy vibratory signaling before contact is made with the female (Robinson & Robinson 1980; Barth 1990; Arnqvist 1992; Robertson & Adler 1994), and male spiders may advertise their quality using these vibrations (Coyle & O'Shields 1990; Mappes et al. 1996; Parri et al. 1997). Vibrations of the substrate and the vigor of the display may indicate to the female the size of the courting male and his physical condition (e.g., Kotiaho et al. 1996; Rivero et al. 2000; Singer et al. 2000; Maklakov et al. 2003). We conducted experiments in which courtship was interrupted before copulation, and previously courting males or females were then paired with naive (non-courting) mates. By this method, we could compare the behavior of males to naive, virgin females and to females presumed to have been sexually stimulated. The specific predictions for each of the two hypotheses are described below.

METHODS

We collected juvenile and subadult widow spiders, *L. pallidus* from sites around Beer Sheva in July–October, 1998; March–April and June–August, 1999; and June–August, 2000 and 2001. Spiders were taken into the laboratory at the Sede Boqer Campus of Ben-Gurion University, Israel, and kept at 26–28° C, approximately 30% relative humidity and a 10:14 light:dark cycle, similar to the prevailing hours of light and dark for the time of year (March–April). Females were housed in plexiglas cages (15 × 30 × 20 cm) with thin branches placed in one corner, on which the nest and web were constructed. We fed the females twice a week with nymphs of either locusts (*Locusta* sp.) or crickets (*Acheta domestica*) and fed the males once a week with either first instar locusts or adult fruit flies (*Drosophila melanogaster*). We eonducted all experiments after the females had rebuilt their nests and molted to adulthood. Recently molted virgin males and females were selected at random with respect to body size.

In order to determine which of the hypotheses, cooperation or conflict between the sexes, better explains the courtship display we first documented courtship in *L. pallidus*, which had not been described previously.

Male courtship display.—We placed *L. pallidus* males (n = 28) individually on the cage inner wall near the capture webs of conspecific virgin females during the morning hours. Observations were begun when the male moved onto the female's web and lasted for two hours or less if the male entered the female's nest, climbed onto the dorsal side of her abdomen, and then moved to the ventral side into a mating position. We defined the behavioral patterns of the display, and recorded the sequence and starting time of each pattern, for each of the males as well as the response of the courted female. We noted the starting time of each behavioral pattern, rather than its duration, because the behaviors were often performed intermittently and short bouts of different components alternated with one another. The time when a male climbed onto the dorsal side of the female's abdomen we designated as the "commitment step"; after this step, 96.4% of the males copulated successfully.

Testing the hypotheses: cooperation versus conflict of interest.—According to the *cooperation hypothesis*, the male's display is aimed at stimulating the female. Thus, the female is expected to signal to the male (e.g., by behavioral or pheromonal cues) when she is ready to mate, and the courting male is expected to respond by entering the nest, mounting the female and copulating. The *conflict of interest hypothesis* suggests that the display provides the female with information about male quality. Thus, the female imposes an energetically costly display on the male as a test of his quality or physical condition. Under this hypothesis, the female is expected to reject males that do not display or whose display is in some way inadequate.

Rejection of a male by the female involves plucking or jerking the web and even chasing the male from the nest entrance. Acceptance of a male, however, does not usually involve an overt behavior. Therefore, we used the response of the male as an indication of a female signal to go on to the next behavioral pattern in the display.

In the following (i–iii) experiments we used males and females collected as juveniles during 1998–1999. In experiment (iv) we used males and females collected during 2000–2001. In all experiments, a virgin male was placed on the inner wall of the cage containing an adult female with her nest and capture web. We observed the pair for three hours or until the male mounted the female's abdomen, and we recorded the times from the start of the courtship until the male a) entered the female nest and b) reached the female abdomen. We conducted three experiments (i–iii) with virgin males and females, in which males and females were either naive, or had already engaged in courtship (experienced). The fourth experiment (iv) compared the behavior of virgin males to virgin or mated females.

We compared durations until the male entered the female's nest (duration of courtship on the web) and until he mounted the female's abdomen (total courtship duration) for the first three tests (i–iii) described below, and used Tukey's post hoc test for pairwise comparisons of mean durations. The data were tested for normal distribution (Liliefors test of the residuals, Systat 10, 2000). ANOVA was used to test for differenees among the means for data that were distributed

normally, and Kruskal-Wallis test for data that were not normally distributed. In the fourth experiment (iv) we used a Mann-Whitney U test to compare the response of males to virgin and mated females.

(*i*) *Naive males and naive females*: The courtship of this species is undescribed, so we observed the courtship duration of naive males placed with naive virgin females (n = 20). These data serve as a baseline against which we compare the duration of male displays in the subsequent experiments.

(*ii*) *Naive males and experienced females*: In order to test the likelihood that a signal is transferred from a stimulated female to a courting male, we placed a naive *L. pallidus* male onto a web of a female who was courted, but not mated, by a previous male ("stimulated" female) (n = 11). The time between removal of the first male at the commitment step and introducing the second, naive male to the female was < 2 min. Following the cooperation hypothesis, the naive courting male is expected to receive an acceptance signal from the already-stimulated female. He should thus reduce his courtship effort and enter the nest after only a short display. Following the conflict hypothesis, the female should accept the new male only after a full display on the web; the naive male should be unaware that the female was courted previously and therefore will perform the full display. However, since this imposed scenario is not likely to occur often in nature, a female may mistakenly perceive the second male courtship as an extension of the first male's display. In this case the female is expected to be less aggressive, and the male may subsequently reduce his display, which may result in an intermediate display time.

(*iii*) *Experienced males and naive females*: In order to test the two hypotheses further, we observed the display of a previously courting male presented with a naive female (n = 10). As in the previous experiment, < 2 min lapsed between removing the male and presenting him to a naive female. The cooperation hypothesis predicts that a male encountering a non-stimulated female will start a new, lengthy courting display. The conflict hypothesis predicts that a male that has already courted a female, and thus provided information regarding his quality, will cut short his display. The naive female, however, is expected to reject the male until he performs a full display on her web.

(*iv*) *Naive males and mated females*: We compared the courtship duration of naive males presented with mated (n = 20) and with virgin (n = 27) females. The spiders were observed for three hours. Males were removed from the cage after climbing on the female abdomen, and the time was recorded. A week before the experiment, males were left with females for 24 h in order to obtain mated females. We regarded the female as having mated if, two months later, at least one egg sac was constructed and the spiderlings hatched. The cooperation hypothesis predicts that a male will display similarly to a mated or a virgin female, since physical stimulation is required by the female, independent of her mating status, before mating can take place. Assuming first-male sperm priority (Austad 1984; Segev et al. 2003), the conflict hypothesis predicts that virgin females should be choosier than mated females, forcing the males to perform a lengthy display.

The predictions of the two hypotheses for each experiment are summarized in Table 1.

Table 1.—Predicted behavior of males in four experiments for each of the two hypotheses proposed: I. Cooperation (mutual stimulation), II. Conflict and female choice for male quality.

	Test	I. Cooperation	II. Conflict
i)	Naive male and naive female	Display	Display
ii)	Naive male and experienced female	Reduce display	Display
iii)	Experienced male and naive female	Display	Reduced display
iv)	Naive male and mated female	Display	Reduced display

RESULTS

The courtship display.—The courtship display of *L. pallidus* males is similar to other *Latrodectus* species (Kaston 1970; Anava & Lubin 1993). The entire courtship is exceedingly long: of 28 *L. pallidus* males observed courting virgin females, only 19 completed the courtship display on the web and entered the nest in less than two hours (mean ± SD, 109.21 ± 19.07 min).

Most of the display took place on the web, before entering the nest. The final display was performed inside the nest before copulation. The courtship on the female's web consisted of a series of complex movements performed in a specific order (Fig. 1). The male entered the female's web via the frame threads and proceeded to walk on the web, while laying his own dragline threads and disconnecting the lines of the female's web. He cut the thick attachment lines to the substrate, the sticky threads attached to the ground, as well as the threads of the platform and barrier web, and wrapped the web silk into small bundles, which he suspended from the female's threads, usually near her nest (web-reduction behavior: Watson 1986; Anava & Lubin 1993). Finally, he performed small and rapid movements (jerking and abdomen vibrations) on the female's web before entering the nest, followed by climbing on the dorsal side of the female's abdomen and then moving to her ventral side. Jerking movements were sometimes performed inside the nest as well, just before the male climbed on the female's abdomen to attempt copulation. While on the female's ventral side, the male drummed with his pedipalps on and near the female's genital openings (epigynum), and finally inserted a pedipalp into the female genital opening. A female might chase the male

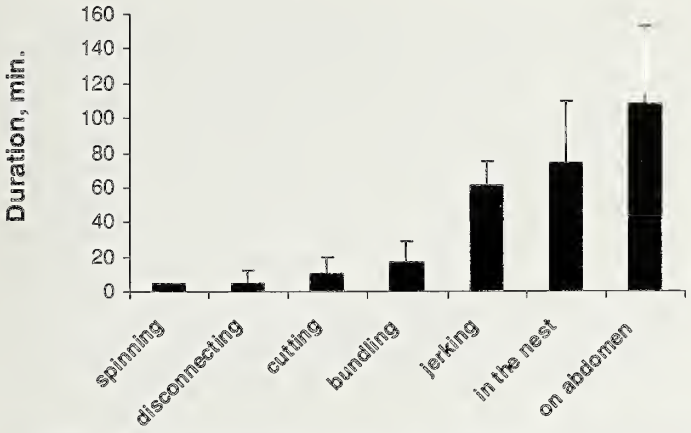

Figure 1.—The sequences of behaviors constituting the courtship display of male widow spiders *Latrodectus pallidus* (n = 19). Shown are mean ± SD of the first occurrence (in minutes from the start of the display) of each behavior component. Note that some components were performed in alternation (e.g., disconnecting capture-web threads, cutting of frame threads and bundling of threads). "On abdomen" refers to climbing onto the dorsal side of the female's abdomen.

away at various stages, and the male would then resume courting on the web, outside the nest. Once the male was on the female's abdomen, however, copulation usually followed. We referred to the male mounting the female's abdomen as the *commitment step*, and in the following analyses we have taken this step as an indication of the female's acceptance of the courting male.

Cooperation versus conflict of interest hypotheses.—*Duration of courtship on the web:* The durations of male courtship prior to entering the female's nest were not normally distributed. The display time before entering the female's nest differed significantly among the four tests (Kruskal-Wallis test: H = 21.601, $P < 0.001$, n = 38). In each of the different experiments, some males did not enter the nest within three hours or did not reach the commitment step during the observation time. These males, fewer than 20% of each experiment, were excluded from the statistical analyses.

Naive males that courted naive females (i) (n = 18), displayed for 77.67 ± 39.91 min (mean ± SD) before entering the nest. Naive males that were placed in cages of virgin females that had been courted previously by another male (ii) (n = 10), displayed for 68.80 ± 26.26 min before first entering the nest. All males (n = 10) that were transferred to cages of naive females after reaching the commitment step in the nest of another female (iii) immediately attempted to enter the second female's nest (display duration 3.0 ± 1.94 min), and all were chased away by the female.

Total courtship duration: There was no significant difference among the different experiments in overall courtship time until reaching the commitment step on the female's abdomen (ANOVA: $F_{2,28}$ = 2.862, P = 0.08). Naive males that courted naive females (*i*) (n = 16), reached the commitment step after a total display duration of 101.73 ± 23.55 min. When paired with previously courted virgin females (*ii*) (n = 8), the display duration of naive *L. pallidus* males until the commitment step, was 72.5 ± 16.66 min. Virgin males that had previously courted an experienced female and were then placed in a cage with a naive female (*iii*) were chased out of the nest by the female when they first tried to enter the female's nest. One of these rejected males did not resume courting, and an additional male did not reach the commitment step within three hours. All other males (n = 8) resumed courting on the web and repeated all behavioral patterns in the normal order (Fig. 1). For these males, courtship duration until the commitment step was 86.57 ± 24.91 min.

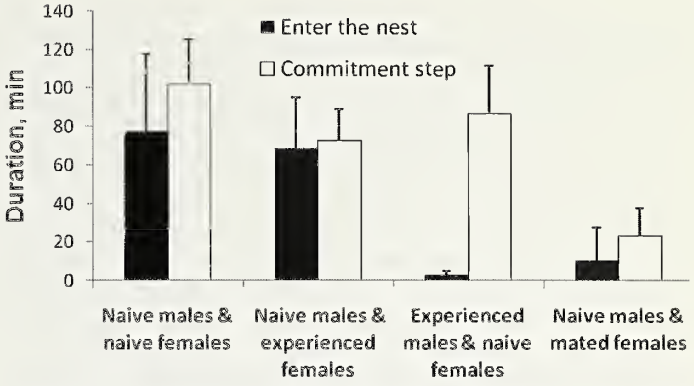

Figure 2.—Durations (mean ± SD) of male courtship on the web until entering the female's nest (black bars) and of the total courtship display until the commitment step (white bars) for each of the four tests.

Virgin males tested with mated females (iv) did not cut the thick attachment threads of the female's web or bundle them in front of her nest. Instead they briefly laid dragline silk on the female's web, cut some of the thin web threads and rapidly entered the female's nest and climbed on the her abdomen, jerking briefly just before entering the nest (duration until entering the nest 10.41 ± 17.38 min, until commitment step 23.33 ± 14.62, n = 15). Naive males courting virgin females (n = 21) performed the typical courtship sequence until entering the female nest (60.05 ± 33.1 min) and reached the female's abdomen (90.7 ± 35.6 min) after a significantly longer time (Mann-Whitney U test: U = 284.5, n = 36, $P < 0.001$ and U = 308.0, n = 36, $P < 0.001$, respectively). The duration of the courtship display of males in all of the experiments are summarized in Fig. 2.

DISCUSSION

The results of the experimental manipulation of male and female experience suggest that the male's courtship display is better explained by the hypothesis of conflict over mating interests between the sexes than that of male and female cooperation to sexually stimulate the female. We discuss the two hypotheses suggested in the light of our results.

Cooperation.—In some spider species, the female signals her acceptance by adopting a receptive posture (Robinson & Robinson 1980), whereas in others females indicate receptivity by remaining stationary (Forster 1982). In the widow spider *L. pallidus*, the females draw away from the nest wall towards its center, allowing the male the space needed to approach the ventral side of her abdomen. Testing the hypothesis of cooperation aimed at stimulating the female, we predicted that females that were previously stimulated would signal their readiness to mate. Upon perceiving the signal, a male should respond by ceasing his display and entering the nest. However, we found that males did not change their display when they encountered previously courted females (test ii). Apparently,

they did not identify these females as receptive, and their courtship duration was not statistically different from that of males courting naive females (test i). In addition, a male that was interrupted during his display at the commitment step and then transferred to a cage with a naive female (iii) immediately attempted to enter the female's nest and proceed with his display from the point at which it was interrupted. Thus, we suggest that the male failed to receive a signal from the female indicating that she was not stimulated to mate. Finally, if courting functions to stimulate the female physically before mating, previously mated females presented with a male several days after their first mating should not differ from virgins in requiring a stimulating courtship display. Contrary to this prediction, however, we found that males shortened their display when they encountered mated females.

Conflict over mating interests.—The courtship display of the male widow spider *L. pallidus* is lengthy and consists of behaviors such as web vibration (jerking) and cutting and bundling of silk that are likely to be energetically costly. The entire process may take more than two hours before the male enters the nest and a total of four or more hours before he copulates with the female (A.R. Harari, personal observation). At each stage of the display, females may gain information concerning the male's quality.

Our experiments revealed that virgin females prevented males from mounting and copulating if the males had not performed all parts of the display, and naive males courted virgin females regardless the female's previous experience. These results suggest that males expect to be accepted by females only after displaying the full courtship, and that a female accepts a male only after he completes a full display on her web. We tested both sides of the coin by observing whether a naive male courted a female immediately after she had been courted by a previous male up to the commitment step (ii). In this experiment, males engaged in the full courtship display, suggesting that they did not receive any cue from the female. In the reciprocal experiment (iii), a naive female was courted by a male whose courtship with a different female was interrupted at the point of entering her nest. The male's response, to continue from the point that he had stopped, suggests, again, that he initially received no cue from the female. The female's aggressive response, however, suggests that she had not had an opportunity to assess the male's quality and therefore rejected his attempt to enter the nest. Overall, we interpret our results as indicating that the male's display is under female control, such that males that attempt to shorten the display are prevented by virgin females from proceeding with close-range courtship inside the nest, mounting and copulation.

Additional support for the hypothesis of a conflict over mating interests and female control over the length of the male's display comes from the results comparing the male's display to a mated or virgin female. Female widow spiders may mate with more than one male (Anava & Lubin 1993; Andrade 1996; Segev et al. 2003; Segoli et al. 2006). We found that males of *L. pallidus* shortened their display significantly when courting mated females, a behavior that is expected if there is first-male sperm priority, and thus, a lower probability of fathering the offspring from a second or later mating. Segev et al. (2003) showed evidence for first-male sperm priority in *L. revivensis*, as is the case in many other entelegyne spider species that have been tested (Christenson & Cohn 1988; Watson & Lighton 1994; Singer & Reichert 1995; Snow & Andrade 2005, but see e.g., Schneider at al. 2000 for a case of mixed paternity). In *L. pallidus* (Segoli et al. 2006) as well as in other *Latrodectus* species (Levi 1959; Bhatnagar & Rempel 1962; Kaston 1970; Foelix 1996; Berendonek and Greven 2002; Snow et al. 2006; Segoli et al. 2008), the tip of the male's embolus is often broken during copulation and becomes lodged in the insemination duct or inside the spermatheca. The presence of the broken embolus tip may act as a mating plug and may reduce the likelihood that a second male will successfully inseminate the female (Berendonck & Greven 2002; Segoli et al. 2008). Thus, if the first male is aecepted only after a stringent test and the contribution of the second male to the clutch is limited, a mated female may be less choosy with subsequent males. Accepting a second male with little courtship could be a bet-hedging strategy, as suggested for *Neriene litigiosa* (Keyserling 1886) (Linyphiidae) by Watson (1991b).

Our results indicate that males are able to distinguish between virgin and mated females (as shown in *L. hasselti*, Stoltz et al. 2007) and reduce their display effort to the latter. Pheromones produced by the female have been shown to play an important role in mate attraction (e.g., *L. revivensis*: Anava & Lubin 1993) and may also provide a means of assessing female reproductive state on the web (Papke et al. 2001). However, it will always be advantageous for males to shorten the display duration and reduce its cost if the female will allow it. In courting virgin females, males frequently attempt to enter the nest and are repeatedly chased off by the female to continue their display on the web. This behavior suggests that males repeatedly test the female's aggressive intentions during courtship; virgin females reject males that have not met a criterion, whereas mated females accept a second male more readily.

In conclusion, our experiments suggest that the costly display of the widow spider *L. pallidus* is unlikely to function as physical stimulation of the female to mate. Rather, the courtship display in this species is likely a result of a conflict of interests, with the female imposing a long, vigorous and energetically costly display in order to test the male's quality.

In recent years, the view of reproduction as a cooperative effort has been challenged by increasing evidence for conflicting interests between the sexes (Dawkins 1976; Parker 1979; Holland and Rice 1998; Zeh and Zeh 2003). Although both parents share an interest in maximizing the fitness of their offspring, they often have conflicting interests in the amount of reproductive effort (Parker 1979). This conflict begins with the investment in the size of male and female gametes (anisogamy, Trivers 1972) and continues with the conflict over the number of matings (Bateman 1948). As a consequence, females are expected to be choosy, selecting some males and rejecting others based on their phenotypie traits (Andersson 1994; Arnqvist and Rowe 2005). This scenario may lead to the complex and lengthy male display in various spider species, including *L. pallidus* (Kaston 1970; Ross & Smith 1979, Lubin and Anava 1993; Forster 1995). The fixed components observed in the display of *L. pallidus* and other *Latrodectus* species (e.g., Lubin and Anava 1993) can be viewed in the light of the known predatory and cannibalistic nature of the genus (Elgar & Schneider 2004) and may be aimed at appeasing the females by providing cues for species recognition (Ryan 1985; Andersson 1994). The length of the display and its energetic cost, however, may have evolved as

a consequence of female choice for energetically displaying males. The results of our experiments support the latter view.

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Redescription and transfer of *Geolycosa grandis* (Araneae, Lycosidae) to the genus *Hogna*

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Abstract. *Geolycosa grandis* (Banks 1894) (Araneae, Lycosidae) is redescribed, and illustrations are provided for the first time. We propose that *Geolycosa grandis* be transferred to the genus *Hogna* Simon 1885. *Hogna permunda* (Chamberlin 1904) is synonymized with *H. grandis*. Notes are given on distinguishing *H. grandis* from similar species including *H. helluo* (Walckenaer 1837), *Allocosa georgicola* (Walckenaer 1837), and *H. aspersa* (Hentz 1844). Information on *Lycosa permiana* Scheffer 1904 is examined and the species is declared *nomen dubium*.

Keywords: Wolf spider, taxonomy, synonymy, new combination, *nomen dubium*

Geolycosa grandis (Banks 1894) is a large wolf spider found throughout the north central Great Plains of North America. Although a large wolf spider, arachnologists have routinely overlooked or misidentified the animal almost from the time of its original description. Banks's original 1894 description provides an adequate depiction of *Lycosa grandis* for correct identification. However, likely due to a lack of illustrations, the species has remained obscure to date. Chamberlin, who described the spider as *Lycosa permunda* in 1904, had apparently either not read or misunderstood Banks's original description. Chamberlin included both *L. permunda* and *G. grandis* (as *Lycosa grandis*) in his lycosid revision of 1908; however, Chamberlin indicates that he had not examined Banks's type specimen of *L. grandis* from Colorado; he only had studied a specimen he identified as *L. grandis* from Baja California. It is not known what species Chamberlin identified as *L. grandis* from Baja as the specimen referred to could not be located. Research conducted for this paper indicates that the range of *L. grandis* does not extend that far south.

We contacted any institution that might have possession of the type *L. permunda*, including the American Museum of Natural History, United States National Museum, Museum of Comparative Zoology at Harvard University, University of Kansas Natural History Museum, and Brigham Young University Museum. In his description, Chamberlin makes note of a spider having a carapace length over 10 mm and a "pale narrow median line extending backward from first eye row, widening abruptly in front of dorsal groove." He also mentions a clear abdominal pattern, light venter, and an epigynum as in *Hogna helluo* (Walckenaer 1837). The lack of another spider having these characters in the Colorado/Kansas area indicates it is *G. grandis*.

The holotype male of *Lycosa grandis*, deposited at the Museum of Comparative Zoology, was found in a vial with a male *L. coloradensis* Banks 1894 and a female *Agelenopsis aperta* Gertsch 1934. It is not clear why these specimens are together in the type vial. *Lycosa grandis* was transferred to *Geolycosa* by Roewer (1955); however, the reason for this transfer was not made clear.

The proposed transfer of *Geolycosa grandis* into the genus *Hogna* is based on comparisons with the generotype *Hogna radiata* (Latreille 1817) from Montpellier, France, provided by Charles Dondale (the holotype is presumed lost), as well as comparisons with other North American representatives of the genus *Hogna*. The species is placed into *Hogna* based on similarities of the palpal structure, specifically the similar median apophysis shape, the two-part terminal apophysis, and the palea shape. The species also shares similarities in the internal and external epigynal structures, specifically the spermathecal shape, as well as similarities in the carapace shape, eye location, and leg length. Illustrations of *H. grandis* are provided here for the first time.

Justification for the removal of *H. grandis* from *Geolycosa* is also supported by the lack of characteristics diagnostic for *Geolycosa*, specifically the lack of a sloped carapace, a defining character for the genus *Geolycosa* (Wallace 1942; Dondale & Redner 1990). In addition, the species differs behaviorally. According to Wallace (1942), spiders of the genus *Geolycosa* spend practically their whole existence in a burrow and females rarely leave the burrow. In contrast, *G. grandis* is a non-obligate burrower and the females spend considerable time roaming outside the burrow.

METHODS

Illustrations were made from digital photographs taken with an Olympus U-CMAD3 digital camera mounted on an Olympus SZX12 stereo-microscope. Scanning electron micrographs were taken with a Hitachi TM-1000 tabletop microscope. All measurements are in millimeters. Specimens used for this study are housed in the arachnological collection at the Denver Museum of Nature & Science, the University of Nebraska Museum, and the Museum of Comparative Zoology at Harvard University. Specimens of comparison species are also housed in the DMNS collection.

Abbreviations.—MA = median apophysis; TA= terminal apophysis; MS = median septum; PLE = posterior lateral eyes; PER = posterior eye row; AME = Anterior median eyes; AMNH = American Museum of Natural History; BYU = Brigham Young University; DMNS = Denver Museum of Nature & Science; KU = Kansas University; MCZ = Museum of Comparative Zoology; USNM = United States National Museum, Smithsonian Institution.

TAXONOMY

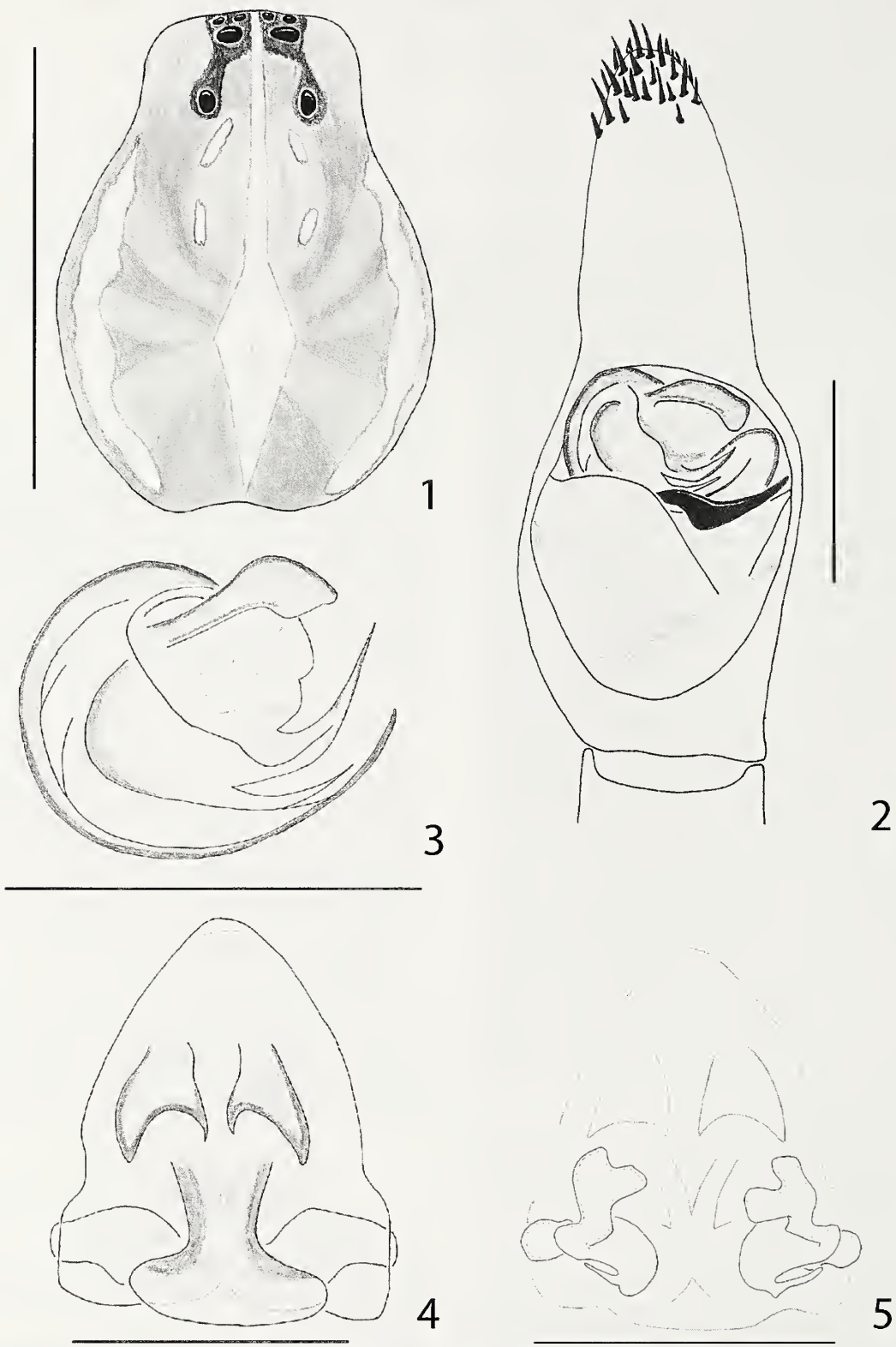
Hogna grandis (Banks 1894) new combination

Figs. 1–6

Lycosa grandis Banks 1894:49; Chamberlin 1908:229.
Lycosa permunda Chamberlin 1904:286; Chamberlin 1908:233, NEW SYNONYMY.
Hogna permunda Roewer 1955: 259; Platnick 2008.
Geolycosa grandis (Banks 1894) Roewer 1955:244; Platnick 2008.

Type material.—*Holotype:* USA: COLORADO: *Larimer County*, Fort Collins (40.35°N, 105.05°W, elev. 1525 m), male, no date, MCZ. Examined.

Holotype male and paratype female: Lycosa permunda Chamberlin 1904: USA: Kansas, no date. Unable to locate specimens.



Figures 1–5.—*Hogna grandis* (Banks 1894). Figs. 1–3. Male (DMNS. ZA11887): 1. Dorsal view of carapace; 2, 3. Ventral view of palpus; p = palea, MA = median apophysis. Figs. 4,5. Female (DMNS. ZA.9572): 4. Ventral view of epigynum; 5. Dorsal view of spermathecae; bc =basal chamber, ll = lateral lobe, tc = terminal chamber, ss = spermathecal stalk. Scale line = 1 m.

Figures 6, 7.—Scanning electron micrographs of male palpus. 6. *Hogna grandis* (Banks 1894); 7. *Hogna helluo* (Walckenaer 1837).

Other material examined: 17 males and 25 females; USA: COLORADO: *Arapahoe County*, 1M, 21309 E. Belleview (40.58°N, 106.23°W), no date, B. Parshley, DMNS ZA.12744; 1F, Aurora (39.65°N, 104.75°W), 20 August 2001, B. Shipley, DMNS ZA.12740; 1F, I-70, 0.5 mi (0.8 km) N of Elbert Co. Line (39.58°N, 104.02°W), 30 August 2004, H. Guarisco, DMNS ZA.9572; *Boulder County*, 1F, 2 mi (3.2 km) E. Marshall (39.96°N, 105.23°W), 16 April 1961, B. Vogel, DMNS ZA.2128; 1M, Boulder (40.02°N, 105.31°W), 31 July 1961, D. Ward, DMNS ZA.2129; *Delta County*, 1M, North entrance Crawford State Park (38.70°N, 107.60°W), 31 July 1999, C. Swinney, DMNS ZA.12738; *Denver County*, 1F, Washington Bay in Denver near Southwest Plaza (39.83°N, 105.20°W), October 1998, D. M. Endricks, DMNS ZA.12739; *Douglas County*, 1M, 10615 Jewelbenny Trail, Highlands Ranch (39.32°N, 104.93°W), 17 August 2006, no collector given, DMNS ZA.13376; 1F, 6414 E. Dutch Creek St., Denver (39.71°N, 104.99°W, 12 August 2000, N. & J. Tuchton, DMNS ZA.12737; 1F, Sharptail Open Space, Louviers (39.45°N, 105.05°W), 27 May 2005, B. Morrison, DMNS ZA.14252; *El Paso County*, 1F, Bilerest Terrace (38.88°N, 104.76°W), 16 July 2001, A. Broughton, DMNS ZA.11888; *Jefferson County*, 1M, 9797 West Ohio Avenue, Lakewood (39.70°N, 106.11°W), August 2001, E. House, DMNS ZA.12721; *Larimer County*, 1M, 1756 Haase Court, Berthoud (40.31°N, 105.81°W), 18–20 August 1999, P. Phillips, DMNS ZA.12731; 1F, 2120 Bridgefield Ln, Ft. Collins (40.56°N, 105.10°W), 29 August 2004, J. Enstrom, DMNS ZA.13770; 1F, 24205 Colorado Ave, Loveland (40.37°N, 105.08°W), 30 July 1999, D. Goldade, DMNS ZA.11840; 1F, 7894 Little Fox Lane, Wellington (40.70°N, 105.00°W), 14 September 2000, M. Payew, DMNS ZA.11883; 1F, Dixon Reservoir (40.55°N, 105.14°W), 24 May 2000, D. Chlebown, DMNS ZA.11886; 1F, Environmental Learning Center (40.57°N, 105.01°W), 25 September 1999, J. M. Diez, DMNS ZA.11882; 1F, Fort Collins (40.58°N, 105.11°W), 30 August 1973, W. D. Frank, DMNS ZA.11875; 1M, Same locale, 10 August 1970, W. D. Frank, DMNS ZA.11880; 1M, Same locale, 24 November 1980, W. D. Frank, DMNS ZA.11881; 1M, Same locale, 9 August 1987, D. Johnson, DMNS ZA.11872; 1F, Same locale, 13 Jun 1989, B. Holter, DMNS ZA.11797; 1M, Same locale, 4 March 1985, W. D. Frank, DMNS ZA.11879; 1F, Same locale, 15 July 1980, W. D. Frank, DMNS ZA.11877; 1F, Same locale, 4 January 1971, W. D. Frank, DMNS ZA.11876; 1M, Same locale, 23 September 2001, L. Sandner, DMNS ZA.12698; 1F, Same locale, 11 September 1990, no collector given, DMNS ZA.11874; 1M 1F, Same locale, 1 September 1977, W. D. Frank, DMNS ZA.11873; 1F, Same locale, 18 September 1973, W. D. Frank, DMNS ZA.11878; 1F, Same locale, 22 October 1982, D. Clarkson, DMNS ZA.11798; 1F, Loveland (40.40°N, 105.11°W), 10 October 1967, W. D. Frank, DMNS ZA.11885; 1M, Same locale, 7 August 1990, Kilburn, DMNS ZA.11884; 1M, Loveland (40.40°N, 105.11°W), no date, A. Randall, DMNS ZA.12718; *Weld County*, 1M, Bones Galore Paleo Site, Pawnee National Grassland (40.73°N, 103.80°W), 15 August 2001, T. Hiester, DMNS ZA.11887; 1F, Bones Galore Paleo Site, Pawnee National Grassland (40.75°N, 103.80°W), 18 August 2000, T. Hiester, DMNS ZA.12722; KANSAS: *Montgomery County*, 1F, 1 mi (1.6 km) E Havana Reservoir (37.22°N, 95.71°W), 25 July 1974, no collector given, DMNS ZA.11306; *Wyandotte County*, 1F, Mission Grade School 2 mi (3.2 km) N. Bonner Springs (39.06°N, 94.88°W), 29 September 1977, R. Huggins, DMNS ZA.11308; 1M, Mission Grade School, 3 mi (4.8 km) N. Banner Springs (39.16°N, 94.83°W), 29 September 1977, R. Hugging, DMNS ZA.11841; WYOMING: *Campbell County*, 1M 1F, Gillette (44.29°N, 105.50°W), June 2005, no collector given, DMNS ZA.14260.

Diagnosis.—*Hogna grandis* can be diagnosed by its size, total length over 20 mm; carapace length about 10 mm; a clear median band on the carapace originating from between the AME and expanding just anterior to and around the fovea, forming a diamond or triangle shape; undulating sub-marginal bands; light marks just behind the PLE (Fig. 1); and a patterned abdomen. Male *H. grandis* specimens can be separated from *H. helluo* and *A. georgicola* by the short laterally directed MA (compare Figs. 6 and 7), and from *H. aspersa* males by the color pattern, and a weak or absent sclerite on the palea (Figs. 2, 3). Female *H. grandis* can be separated from *H. aspersa* by a narrower inverted T-shaped MS, which is no wider than one of the epigynal hoods. They can be separated from *H. helluo* by a shorter MS stalk and thicker MS base, and from *A. georgicola* by a less curved atrium and thicker MS base which forms a gradual curve from the stem to the base without depressions along the curve. The bilobed terminal chamber of the spermathecae of *H. grandis* can be used to separate it from female *A. georgicola* and *H. aspersa*.

Description.—*Male (type):* Total length 21.80 mm, carapace length 11.40 mm, carapace width 8.76 mm. Pale median band originating between the AME and abruptly widening just prior to the fovea, then narrowing again posteriorly, creating a diamond shape. Broad pale marginal bands extending from the PER posteriorly, margins of bands often undulating. Light yellow stripe just posterior to each of the PLE, just longer than the length of the PLE, followed by a second light yellow stripe about the same length just posterior to the first stripe. Chelicerae dark brown, endites dark brown with lighter tips. Sternum brown, with a lighter median band. Legs light brown except for tarsus, which is black, covered with hair-like setae. Numerous macrosetae present. Abdomen with dark heart mark followed by several chevrons, each ending with a light mark. Venter light brown, covered with many scattered dark spots. Cymbium with 14 macrosetae at tip. Embolus originating from the distal area behind the palea and smoothly curving until terminating on the retrolateral side below the end of the upper TA sickle. Palea with a distally located, laterally directed weakly sclerotized area. MA

Figure 8.—Collection localities of *Hogna grandis* (Banks 1894).

small, triangular, located on the retrolateral side of the palp, projecting laterally, but not beyond the cymbium border. TA double, sickle-shaped.

Males (n = 8): Total length: 18.2–22.0 mm; carapace length: 8.90–12.33 mm; carapace width: 7.2–10.12 mm. Carapace median band (Fig. 1) may terminate in a more triangular shape than diamond. Second set of light marks just posterior to PLE may be hard to see or absent. Chelicerae may appear black. Sternum various degrees of brown from light to dark. Cymbium may have 10–20 thick macrosetae at the tip. Weakly sclerotized area of palea may appear non-scelerotized (Fig. 3). Size of MA consistent (Fig. 2) relative to the rest of the palp. TA lower sickle not always visible (Fig. 3).

Females (n = 11): Total length: 18.2–23.6 mm; carapace length: 11.2–12.6 mm; carapace width: 8.7–9.8 mm; coloration same as male but darker. Marginal bands of carapace thinner, light marks behind PLE often lacking second light mark set. Legs also darker than male. Females have an inverted T-shaped MS with a narrow stem no wider than one of the epigynal hoods. The base of the MS is thick forming a gradual curve from the stem to the base without depressions along the curve (Fig. 4). The spermathecae have a kidney-shaped basal chamber with a lateral lobe not extending anteriorly beyond the basal chamber. The spermathecal stalk has a globular lateral lobe and an oblong and often bilobed terminal chamber (Fig. 5).

Habitat and distribution.—Specimens of *H. grandis* have been collected in the grasslands east of the Rocky Mountains in Wyoming and Colorado and west into the San Luis Valley of Colorado (Fig. 8). There is some discrepancy in the distribution of the species. Chamberlin (1904) lists the locality of *L. permunda* as being in Kansas, but provided no further information. Worley & Pickwell (1931), in their Spiders of Nebraska, had their lycosid spiders identified by Martin Muma; examination by the author (JS, pers. obs.) of all voucher specimens in the UNB museum found that he had mistakenly identified specimens of *H. helluo* (Walckenaer 1837) as *H. (Geolycosa) grandis*. No specimens of *H. grandis* were found in the voucher set; however, not all specimens listed by Worley & Pickwell could be located. Worley & Pickwell also note that *H. (Geolycosa) grandis* is found only in the eastern half of the state, which is peculiar for a species with a type locality of Fort Collins, Colorado.

More recently Dondale & Redner (1990) cite both *H. helluo* and *H. aspersa* (Hentz 1844) as being found in Colorado and Wyoming; however, upon review of specimens from both states held in the DMNS collection, no *H. aspersa* have been found, and only a single *H. helluo* male was collected in Colorado, indicating that ranges for both species occur further east. The DMNS collection consists of specimens collected since 1998, as well as the private collection of Bea Vogel; therefore, the specimens may not have been available to Dondale & Redner. These species can be confused with *H. grandis*, which may explain the discrepancy.

Spiders have been seen using burrows and roaming at night in search of prey, a behavior similar to *Hogna carolinensis* (Walckenaer 1805) (JS, pers. obs.). They also use perches when kept in captivity, another behavior similar to *H. carolinensis*. Burrow depth is variable from 5–10 cm, with adults preferring shallower burrows. Burrow shape is straight with a small cavity at the base in deeper burrows, and more lateral in shallower burrows. Burrow turrets were not observed.

Taxonomic note.—In researching this paper, we examined Scheffer's (1905) description and illustrations of *Lycosa permiana*, which indicate that the spider is not a *Lycosa*, but rather belongs in the genus *Arctosa*. The type specimen of *L. permiana* could not be located at the KU museum or USNM as indicated in this paper. The type specimen could also not be located at the AMNH or MCZ collections and is assumed lost. Based on the illustrations of the dorsum and epigynum showing the median septum gradually widening posteriorly and appearing covered with hairs, as well as the description of the dorsum of the carapace and abdominal pattern with radiating lines and spots rather than a dark heart mark found in *Hogna*, *L. permiana* may be synonymous with *Arctosa emertoni* Gertsch 1934 or *A. rubicunda* (Keyserling 1877) which both occur in Kansas. Because of the uncertainty about which *Arctosa* species it may be, we declare it *nomen dubium*.

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Nephilid spider eunuch phenomenon induced by female or rival male aggressiveness

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Abstract. Plugging of female genitals via male sexual mutilation is a common sexual repertoire in some nephilid spiders (*Herennia*, *Nephila*, *Nephilengys*), but the behavioral pathways leading to emasculation are poorly understood. Recent work suggests that copulating *Herennia* males damage their reproductive organs during copulation and then voluntarily, and stereotypically, remove their pedipalps to become eunuchs. Presumably, such emasculation increases agility allowing the male to better fend off rival males. However, through our observation of male antagonism in *Nephilengys borbonica* (Vinson 1863) in La Reunion (Indian Ocean), we discovered that genital severance involving the entire male palp is induced by a rival eunuch. Additionally, laboratory matings of the same species from Mayotte provide the first observations of female sexual cannibalism in this species, one such forceful copulation termination leading to emasculation of the entire palp. These novel behaviors suggest that mate plugging and the eunuch phenomenon are more plastic repertoires than hitherto thought, and thus our observations add to possible pathways leading to them. Based on our examination of 791 samples of *Nephilengys* spp. from museum collections and of a freshly collected representative sample of *N. borbonica*, we conclude that i) palpal severance is common (50% of males from the wild were eunuchs lacking both palps), but ii) the females (or perhaps subsequent males) must possess a mechanism for removing severed palps from the epigyna (none had a whole palpal bulb), leaving behind only partial, embolic plugs, and iii) the disparity between male palpal damage (50%) and visible mating plugs in females (21%) merits further research as the relative numbers of severed males and plugged females can offer insight into which sex may have the upper hand in an evolutionary arms race.

Keywords: Sexual conflict, emasculation, plugging, behavioral plasticity, Nephilidae, *Nephilengys borbonica*

Mate plugging via sexual mutilation and eunuchs, males with severed sperm- transferring organs (palps), are common sexual repertoires and outcomes in males of the nephilid spiders *Herennia* and *Nephilengys* (Robinson & Robinson 1978; Robinson & Lubin 1979; Kuntner 2005, 2007). The sparse behavioral data suggest that copulating males typically first produce a mating plug consisting of the distal two palpal sclerites stuck in the female reproductive apparatus (epigynum), thereby preventing additional access to the used female copulatory opening by rival males. Then the males voluntarily remove their copulatory organ and continue in a sterile, and more agile state, to mate-guard the female from competing suitors (Robinson & Robinson 1980). Such a stereotyped pathway leading to the eunuch phenomenon was partially supported by our recent work, which i) establishes the phylogenetic pattern of these behaviors as homologous in nephilids (Kuntner et al. 2008, 2009a); all nephilids with eunuchs also produce mating plugs; ii) provides the evidence that severed organs in *Herennia* indeed function as mating plugs (Kuntner et al. 2009b); and iii) confirms that sexual mutilation and palpal severance (or eunuch behavior) in *Herennia* is voluntary as previously reported for *Nephilengys* (Robinson & Robinson 1980). Plugging in *Herennia*, however, is more likely when the female shows aggression towards the male during copulation (Kuntner et al. 2009b).

However, our new observations reveal behavioral plasticity that complicates the apparently clear pattern of voluntary, post-copulatory self-emasculation. We here report the first observations of plugging that involve immediate, forced whole bulb loss in a nephilid. One such case was induced by male-male antagonistic behavior. We observed eunuch male mate-guarding and male-male antagonism in *Nephilengys borbonica* (Vinson 1863) (Nephilidae) in La Reunion (Indian Ocean) revealing that palpal severance may be forcefully induced by the male (eunuch) rival, and that such plugging may involve the entire male palp. Furthermore, laboratory mating observations of *N. borbonica* from Mayotte (Indian Ocean) revealed that female sexual aggressiveness, which may result in sexual cannibalism, may also induce male whole palp severance. Although based only on a few observations, we report on them because novel behaviors were noted that elucidate the ethological repertoires leading to plugging and the eunuch phenomenon, and because behavioral observations on *Nephilengys* sexual behavior are so scarce. To investigate the prevalence of palpal severance and the apparent absence of whole palpal plugs in preserved museum material, we here also report examinations of 791 samples of *Nephilengys* spp. from museum collections and of a freshly collected representative sample of *N. borbonica* from islands in the Indian Ocean.

METHODS

Field observations.—We monitored male-male and male-female interactions in *Nephilengys borbonica* on the Indian Ocean island of La Reunion (France), in a forest at Colorado-La Montagne (20°54′23.5″S; 55°25′29.4″E; 680 m elev.) on 12 April 2008. Our daytime, non-manipulative observations utilized digital photography (Canon EOS 20D with a 50 mm macro lens and macro flash) and voice recording.

Laboratory observations.—We collected juvenile and adult *Nephilengys borbonica* in their natural environment on the Indian Ocean island of Mayotte (France) at Plage Tahiti (12°51′49.0″S; 45°06′39.4″E; 1 m elev.) on 8 April 2008 and

transported them live in foam stopper vials to the Ljubljana laboratory on 10 April 2008. We kept larger spiders in 60 × 60 × 10 cm perspex frames and smaller spiders in smaller plastic containers, at a constant 27° C temperature, 70% humidity, and 12:12 h photoperiod. We misted the webs daily and fed the adult and subadult spiders houseflies and crickets twice a week, and the younger spiders fruit flies or small crickets daily. We used final molting to adulthood as evidence for virginity, but also mated individuals of unknown mating histories (i.e., those collected as adults). Following the experimental mating protocols of Kuntner et al. (2009b), we placed in contact each experimental pair for up to four hours. Before experiments took place, we moved and opened the frames housing the females and kept them undisturbed for the first 60 min, followed by the transfer of a male onto the female web using a fine paintbrush. We used video (Sony HDV 1080i) and voice recording to document behaviors.

Morphological examination.—All adult spiders from mating experiments were examined for genital damage under a Leica MZ16 stereomicroscope using KOH maceration, methyl salicilate treatment, and spermathecal clipping (see Kuntner et al. 2009b). Spermathecal clipping reveals male parts (emboli) hidden within the female genitals (spermathecae and ducts) and thus provides evidence of male plugging. Microscope imaging was done using a Leica DFC420C digital camera. Two of the three spiders (eunuch male and female) from La Reunion and all those that mated from Mayotte are available as vouchers and will be deposited in the collections of the Smithsonian Institution's National Museum of Natural History.

In order to assess the prevalence of eunuchs and external plugs in nature, we examined a representative sample of newly collected *N. borbonica* from Madagascar and neighboring Indian Ocean Islands, including La Reunion. External examination of these samples with a Leica MZ16 stereomicroscope would have easily revealed whole palpal plugs in females and palpal loss in males.

RESULTS

Field observations.—A web contained the host female and two males, of which the larger one was a full eunuch (individual that had severed both palps) and the smaller one was intact with both palps (Fig. 1A). Our observation started when the female was residing in her retreat above the hub, the full eunuch (hereafter "eunuch") resting 10 cm above her and the palped male (hereafter "male") 10 cm below her (Fig. 1A). Showing no apparent courtship behavior, the male approached the female, entered her retreat, climbed on her venter, and attempted copulation. At the same time, the eunuch aggressively pursued the wooing male into the retreat, forcing him immediately to jump off the female and out of the retreat into a free fall (Fig. 1B), stopped by his signal line that was attached to the female web. The eunuch climed on the female, and while remaining on her, she moved out of the retreat (yet showed no aggressiveness to either male), and remained around her web hub. The male returned on his signal line, re-approached the female, climbed on her venter, and resumed copulatory attempts despite continued eunuch aggression. One copulation attempt was apparently successful resulting in left palp insertion (Fig. 1C-arrow). However, eunuch antagonism (not female aggressiveness) forced the male to flee (Fig. 1D), this time inducing the copulating male to leave behind his entire left palpal bulb, stuck in the female epigynal opening (Fig. 1D-inset). The severed palp broke in the typical position between the tibia and the tarsus (Fig. 1E-inset). The male, now half-eunuch, returned facing the female's front (she remained unaggressive) and climbed back onto her venter in a new copulation attempt, this time with his remaining right palp (Fig. 1E, F). Continued eunuch attacks (Fig. 1F) again forced the male to flee, but only to yet again approach the female, which had returned to her retreat (Fig. 1G). Walking by the prosoma and chelicerae of the unaggressive female, the male moved towards the eunuch, now protecting the female epigynum on her venter (Fig. 1H). The eunuch forced the male to withdraw again, this time onto the female dorsum (Fig. 1I), and the eunuch remained by the female epigynum (plugged by the male severed bulb, Fig. 1I-arrow). Apparently irritated by the two males on her abdomen, the female moved abruptly out of her retreat and the eunuch attacked the male by aggressively biting with his chelicerae (Fig. 1J shows them still widely open). The male jumped again, and the eunuch resumed his mate-guarding, keeping close to the female abdomen in her retreat (Fig. 1K).

Laboratory observations.—The first two male-female encounters on 15 May 2008 did not result in copulation. Male 1 (virgin) attempted to mate repeatedly using both palps with female 1 (unknown history), but failed. As predicted from this episode, subsequent examination revealed an embolic plug in each female opening. Male 2 (unknown history) attempted to copulate with female 2 (previously mated as witnessed by a clutch of offspring), but his approach resulted in female's aggression immediately upon contact and in the first recorded case of sexual cannibalism, where she consumed her suitor (video available at www.nephilidae.com). Again, subsequent examination revealed an embolic plug in each epigynal opening.

On 28 May 2008 at 10:07 h, male 1 (still virgin after unsuccessful copulation attempts) and female 3 (virgin, one week after final molt) were placed together. The male, being placed about 15 cm diagonally below the female, started walking away from her for several seconds, then turned around and headed towards the female at the top of the web. He stopped 10 cm before the female and cleaned his legs in the order 1-right, 1-left, 2-right, 2-left, 4-left, 4-right, then continued towards the female and paused for 68 s about 5 cm away from her. He continued towards the female shaking his whole body twice and pausing again with his first legs 0.5 cm away from the female's first legs. The contact (video available at www.nephilidae.com) occurred after 10 min of idleness. The male walked onto the female's venter, tapped and probed briefly with his palps on the epigynum, and inserted his right palp into the right genital opening (ipsilateral insertion), followed by the male's lateral body twist (abdomen to the left). After only a brief copulation (about 4 s, at 10:20 h), the female aggressively terminated it by manipulating the male with her first and second legs, grabbing him in her chelicerae, and holding on and consuming him. During seizure, the male's right palp broke off and remained stuck in the female's genital opening (Fig. 2A). Her devouring of the male continued (video available at www.nephilidae.com) until

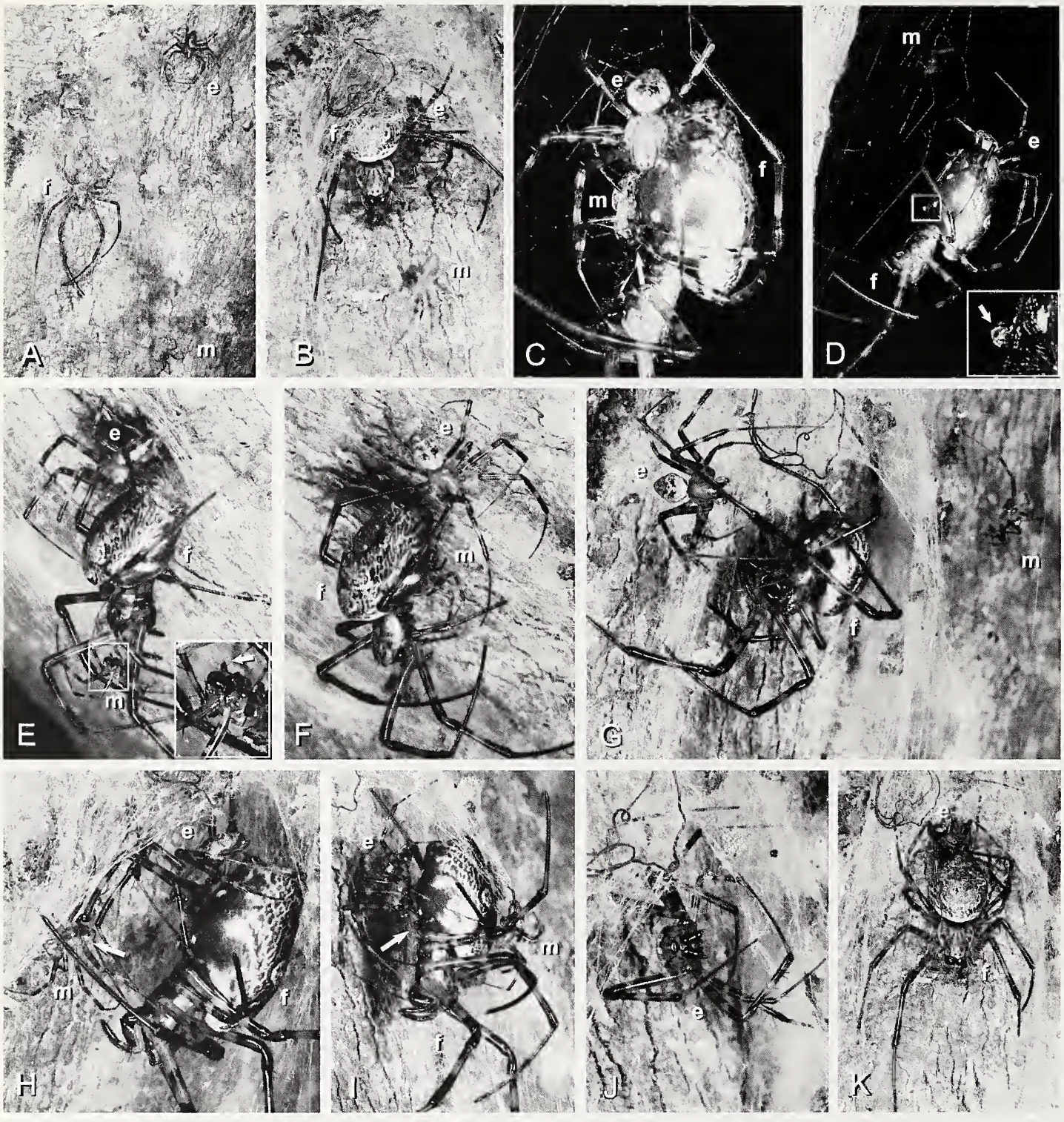

Figure 1.—Male-female and (eunuch) male-male interactions of *Nephilengys borbonica* from La Reunion in chronological order (see text). The male (m) with both palps approached the female (f) (A), but the palpless eunuch (e) repeatedly chased him off her (B–I) and, ultimately, successfully off her web (J–K). A male-male fight during brief copulation (C) resulted in a forceful severance of the male copulating palpal bulb, that remained stuck in the female genital opening (D-inset, arrow, I-arrow) and rendered the sexually active male a half-eunuch (E-inset, arrow, H-arrow).

we interrupted it to secure the male voucher needed for morphological examination. The male lost his right palp at the tibia-tarsus joint (Fig 2B-arrow), as is common in self-castrated eunuch nephilids (Kuntner 2005, 2007).

Morphological examination.—We have previously examined 791 samples containing preserved *Nephilengys* specimens (Table 1, specimen data examined by Kuntner 2007 available at www.nephilidae.com). In *Nephilengys*, all four species are

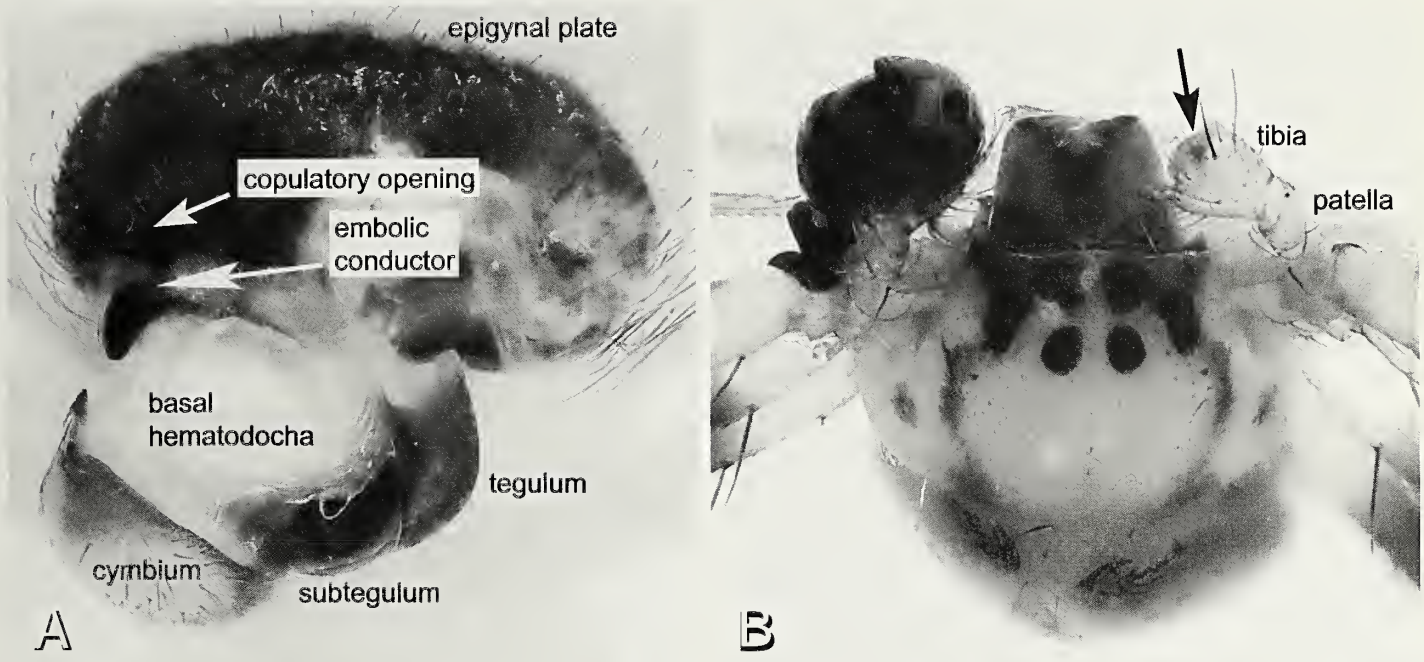

Figure 2.—Outcome of the laboratory mating experiment with *Nephilengys borbonica* from Mayotte. Female forcefully terminated copulation resulting in whole bulb plug in epigynal opening (A) and male becoming eunuch (B) just before being cannibalized by the female. Arrow in (B) indicates the point of palpal breakage.

uniform in gross morphology and behavior, including common eunuchs (Kuntner 2007). However, none of these samples contained females plugged with whole palps.

Table 2 lists the *N. borbonica* specimens examined externally within the representative sample from the 2008 collected material (n = 33 females, 30 males; specimens from mating trials and samples containing only juveniles are omitted). Again, eunuchs were common (50% of males were both palp eunuchs), but while 21% of females had mating plugs in the form of externally visible embolic parts in their copulatory ducts, none of the females had whole palps in their epigyna.

DISCUSSION

The nephilid eunuch phenomenon is not homologous to the voluntary emasculation by theridiid spiders *Echinotheridion* and *Tidarren* (Agnarsson 2006; Kuntner et al. 2008), where the subadult males remove one of their palps before seeking mates. However, increased male agility may explain both phenomena. In these tiny theridiid males, also known for whole palpal plugs and fierce male-male competition (Miller 2007), two large palps reduce the mobility of the male and subsequent emasculation thus facilitates mate searching (Ramos et al. 2004). As sexual cannibalism characterizes these theridiids, males do not have the opportunity to defend mated females. Nephilid males, on the other hand, always start their sexually active post-molting period with a fully functional pair of palps, and become eunuchs after copulation. Hence, unlike their theridiid counterparts, their emasculation serves for post-copulatory agility. In the theridiids, emasculation is highly stereotypical (Knoflach & Harten 2000, 2001). In contrast, our findings here suggest that emasculation does not always follow the same behavioral pattern in nephilids, as previously thought.

Eunuchs are known for post-copulatory mate-guarding and aggressiveness towards rival males (Robinson & Robinson 1978). Our own work on *Herennia* (Kuntner et al. 2009b) has recently confirmed that the eunuch phenomenon typically entails initial sclerite damaging during copulation, followed by voluntary post-copulation palpal removal, corroborating only two previous observations on *Nephilengys* (Robinson & Robinson 1980). However, even if the males of *Herennia* removed their palps themselves after initial mutilation, mutilation itself (resulting in plugging) was more likely when the female was aggressive towards the male during copulation

Table 1.—Numbers of samples containing *Nephilengys* spp. examined morphologically by Kuntner (2007; specimen and depository data available at www.nephilidae.com).

Species	Samples containing female(s)	Samples containing immature(s)	Samples containing male(s)	Total
N. borbonica	86	10	49	145
N. cruentata	360	49	46	455
N. malabarensis	99	8	45	152
N. papuana	24	5	10	39
Total	569	72	150	791

Table 2.—Representative sample of female (f) and male (m) *N. borbonica* collected in nature and examined externally for palpal severance and plugs. Left/right scores are for embolic plug counts in females per opening (0 = no plug; 1 = one plug) and damaged palp in male (u = undamaged; p = palpal severance). More detailed specimen data available from the authors. Note absence of simple embolic severance in males and no half-eunuchs (see Discussion).

Sex	Left	Right	Number of specimens
f	0	0	26
f	1	0	3
f	0	1	2
f	1	1	2
m	p	p	15
m	u	u	15

(Kuntner et al. 2009b). This suggests the possibility that aggressiveness could play a role in inducing palpal severance at various weak points. Although data are sparse, our mating observations suggest that sexual aggression/cannibalism may be common in *N. borbonica* (two out of three mating sequences observed) and, in one case, induced the male to release and leave behind a whole palpal plug in the female opening. Furthermore, the newly observed behavioral pattern of rival-male-induced palpal severance suggests that intraspecific aggression from the female or rival males may induce whole palpal plugging.

Most available observations on nephilid sexual mutilation rely on very few data points (*Nephila* being an exception, see Schneider et al. 2001, 2005; Fromhage & Schneider 2006) and the sexual behavior of *N. borbonica* has not been studied in detail (but see Kuntner 2007). However sparse the data, whole bulb plugging has not been observed before. Certain behaviors such as plugging and bulb loss can be deduced via morphological examination (Kuntner et al. 2008, 2009b). We have examined numerous samples of male and female individuals of *N. borbonica* from its entire range and of *Nephilengys* spp. in total (Tables 1 and 2). Presumably, these examinations would have easily detected any evidence of whole bulb-plugs, yet did not. Similarly, whole bulb-plugs are unknown in *Herennia* or any other nephilid (Kuntner 2005, 2006). The data at hand, if interpreted uncritically, would thus suggest that mating plugs in *Nephilengys* are usually formed by the terminal two sclerites as in most other nephilids (Kuntner 2005, 2007; Kuntner et al. 2008). However, in light of our study, we propose an alternative explanation: that whole palps may be frequently severed and left in the female, but that they subsequently break off at the standard point (the remaining morphological evidence of females would then fail to deteet such involuntary palp loss). Because a palpal bulb consists of the entire palpal tarsus sexually modified in adult males to contain a number of interconnected sclerites and membranes (Fig. 2A), it is thus a relatively large and cumbersome device for the female to keep on her. Additionally, the sexual conflict theory (Arnqvist & Rowe 2005) would predict that it is in the female's interest to resist such a male monopolization mechanism (Kuntner et al. 2009a). We suggest that such plugged females do remove whole palpal plugs (perhaps using their legs or rubbing against the substrate), and that such removal would then result in the common morphological outcome: palpless (eunuch) males, and females plugged only by the male's two terminal sclerites. In fact, the second breaking point between the two sclerites and the remainder of the male palp may be another twist in the male-female conflict story: the male's response to female palp-plug removal. As our preliminary data show, even the two-sclerite plugs seem effective in preventing further matings.

Alternatively, subsequent males, in addition to the female, may be able to remove previous whole palp plugs. Embolic plug removal by the next male is known to occur sporadically in *Nephila fenestrata* (J. Schneider pers. comm.), which is phylogenetically only a few nodes away from *Nephilengys* (Kuntner et al. 2008), although in that species plugs are simpler and generally functional (Fromhage & Schneider 2006). A similar mechanism in *N. borbonica* would then explain the second breaking point in the palpal bulb as another result of sperm competition, in addition to the plugs themselves, rather than a result of sexual conflict between the male and the female. We find this mechanism possible, but unlikely, because it would imply some percentage of females that find no mate subsequent to being plugged, and thus retain the whole palp plug. In our case, this percentage was zero.

Examination of over 800 preserved *Nephilengys* specimens shows high frequencies of female plugging (21%) and male palpal severance (50%). Given that a considerable portion of samples must represent unmated specimens, palpal severance in mated males is likely universal. Our ongoing work (Kuntner, unpublished data) suggests that external genital examination in nephilids underestimates the number of embolic mating plugs (broken emboli may be lodged deep in spermathecae), and thus the real number of plugged females is certainly higher than 21%. Further work is thus necessary to determine if the percentage of plugged females equals the percentage of severed males. Such work should lead to interesting conclusions. If differences in frequencies are confirmed, this would suggest that females are successful in removing mating plugs so that even whole palpal sacrifice by the male may not guarantee his mating success and/or female monopolization. If, however, the numbers of severed males and plugged females are approximately equal, this would imply that male plugs are successful and that males may be winning an evolutionary arms race.

It is worth noting, that the 15 *N. borbonica* eunuchs collected in nature were all full eunuchs, meaning they lacked both palps. Kuntner (2005, 2007) reported also half-eunuchs (males with only one functional palp) in *Nephilengys* and *Herennia*, and we have documented half-eunuchs of *H. multipuncta* in nature (Kuntner et al. 2009b: fig. 2b–c). It remains to be seen whether half-eunuchs are rarer in *N. borbonica* than in *Herennia* and other *Nephilengys* species.

In sum, the newly discovered behavior where sexual mutilation is induced by male or female aggressive behavior and where the mating plug consists of the whole palpal bulb, adds to the documented plasticity in the pathways leading to the nephilid eunuch phenomenon. We conclude that palpal severance in *N. borbonica* is common (50% of males are eunuchs) and that the females are likely able to remove severed palps from their epigyna, resulting in embolic mating plugs.

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On the *charitonovi* species group of the spider genus *Coelotes* (Araneae: Amaurobiidae)

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Abstract. Eight spider species of the genus *Coelotes* from Central Asia and the Near East currently assigned to the *charitonovi* species group are described. Four species, *Coelotes charitonovi* Spassky 1939, *C. juglandicola* Ovtchinnikov 1984, *C. nenilini* Ovtchinnikov 1999, and *C. turkestanicus* Ovtchinnikov 1999 are previously known from both sexes, and four others, *C. caudatus* de Blauwe 1973, *C. arganoi* Brignoli 1978, *C. coenobita* Brignoli 1978, and *C. vignai* Brignoli 1978 are known only from females. The dorsal views of the epigynum of *C. juglandicola*, *C. caudatus*, and *C. vignai* are illustrated and described for the first time.

Keywords: Central Asia, Near East, *Coelotinae*, epigynum, taxonomy

Spassky (1939) described *Coelotes charitonovi* Spassky 1939 from Uzbekistan based only on females. The "group" remained untouched until Charitonov (1969) described *Agelena bucharensis* Charitonov 1969 based only on a male, which has proven to be a junior synonym of *C. charitonovi* (Ovtsharenko & Fet 1980; Ovtchinnikov 1988, 1999). *Coelotes charitonovi* is widespread in Central Asia (Turkmenistan, Uzbekistan, Tajikistan, and Kyrgyzstan) (Ovtchinnikov 1999). de Blauwe (1973) described *C. caudatus* de Blauwe 1973 from Lebanon and Brignoli (1978a) described *C. arganoi* Brignoli 1978, *C. coenobita* Brignoli 1978, and *C. vignai* Brignoli 1978 from Turkey, all based on females. *Coelotes caudatus* is the only coelotine known from the Near East. Although Brignoli (1978b) examined more specimens of *C. caudatus* from this region (2♀ from Col des Cedres; 3♀ from Cedres de Bcharre, and 1♀ from Baskinta), the presence of coelotines in the Near East has not been verified by recent collections, and its occurrence in Lebanon needs confirmation. Ovtchinnikov (1984) described another species, *C. juglandicola* Ovtchinnikov 1984 from Kyrgyzstan, based on specimens of both sexes; spermathecal morphology, however, was neither illustrated nor described. Ovtchinnikov (1999) described two additional species—*C. nenilini* Ovtchinnikov 1999 from Uzbekistan and *C. turkestanicus* Ovtchinnikov 1999 from Uzbekistan, Kyrgyzstan, and Kazakhstan and placed them in the subgenus *Brignoliolus*, together with *C. charitonovi*, *C. caudatus*, and *C. vignai*. Wang (2002) included the species listed above, together with *C. arganoi* and *C. coenobita*, in the *charitonovi* species-group of *Coelotes* based on the anterior, closely-set epigynal teeth, but males were needed in order to support this placement. *Coelotes turkestanicus* was also recorded from the Orenburg Region of Russia, the northernmost limit of its range (Esyunin et al. 2007).

The phylogenetic relationships of the *charitonovi* group species with other coelotines are still unknown. They could be closely related to the *atropos* species group from Europe and Middle Asia in sharing a broad patellar apophysis, a reduced lateral tibial apophysis, a short cymbial furrow, a rounded median apophysis, and a prolaterally originating embolus, but the *charitonovi* group contains species with the patellar apophysis strongly modified and the epigynal teeth distinctly long and closely set.

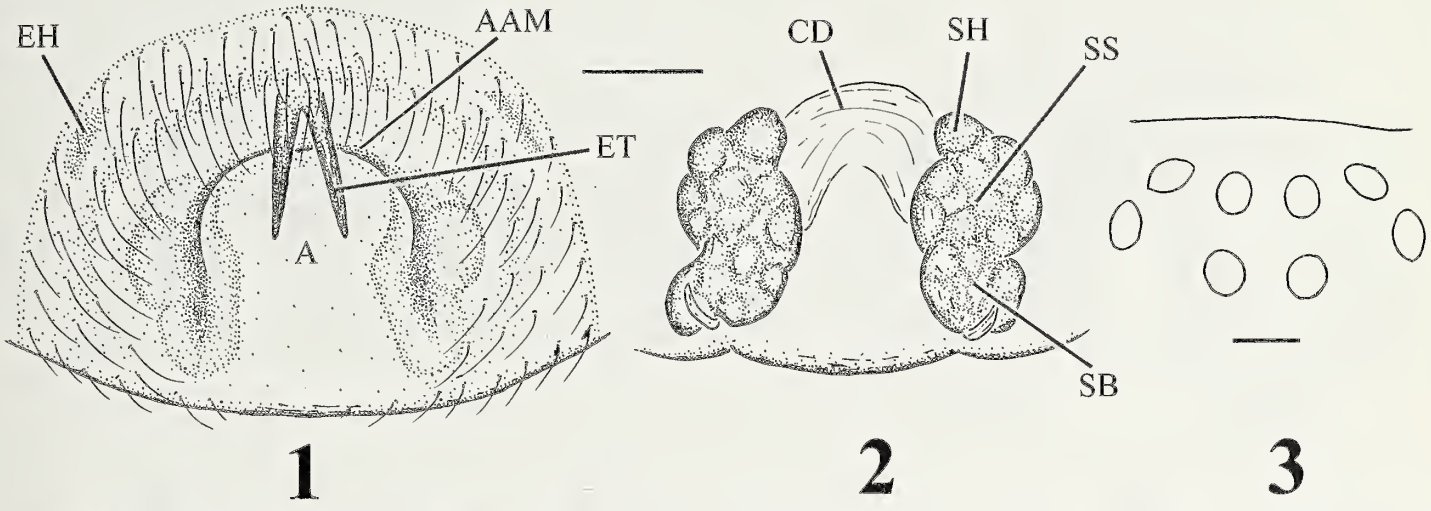
Within the *charitonovi* group, the species are geographically distinctly separated. The four species from eastern Central Asia (*C. charitonivi*, *C.julandicola*, *C. nenilini*, *C. turkestanicus*) might be closely related in sharing the atrium that is situated anteriorly at level of epigynal hoods or anterior epigynal hoods, the elongated spermathecae, and other similarities in the male palp. Unfortunately, data from males were not available for the other four species from western Central Asia and Near East. Their females share the medially or posteriorly situated atrium, the long epigynal teeth, and the more or less broad spermathecae.

In this paper, all species of the *charitonovi* group are redescribed and a key is provided to the species, with particular focus on the genitalic structures. The dorsal views of the epigynum of *C. juglandicola*, *C. caudatus,* and *C. vignai* are illustrated and described for the first time. *Coelotes nenilini*, of which specimens are not available, is described based on the illustrations of Ovtchinnikov (1999).

METHODS

All measurements are in millimeters. Scale lines are 0.2 mm. Terminology used in the text and figures follows Wang (2002). The distribution map was generated using GIS ArcView software, and the specimen files of the species studied can be downloaded from Wang (2009). Due to the limitation of available specimens from this region, this study is based mainly on the examination of type specimens, which were loaned from the following museums: AMNH = American Museum of Natural History, New York (N.I. Platnick); MHNG = Muséum d'histoire naturelle de Genève, Switzerland (B. Hauser); MNHN = Muséum National d'Histoire Naturelle, Paris (C. Rollard).

Abbreviations used in the text are: AAM–anterior atrial margin; AME–anterior median eyes; ALE–anterior lateral eyes; AS–atrial septum; C–conductor; CD–copulatory duct; CDA–conductor dorsal apophysis; CL–conductor basal lamella; CY–cymbial furrow; E–embolus; EB–embolic base; EH–epigynal hood; ET–epigynal tooth; FD–fertilization duct; LAM–lateral atrial margin; LTA–lateral tibial apophysis; MA–median apophysis; PA–patellar apophysis; PAM–posterior atrial margin; PLE–posterior lateral eyes; PME–posterior median eyes; RTA–retrolateral tibial apophysis; S–spermathecae; SB–spermathecal base; SS–spermathecal stalk; SH–spermathecal head; ST–subtegulum; T–tegulum; TS–tegular sclerite.



Figures 1–3.—*Coelotes arganoi* Brignoli 1978, female holotype from Erzincan, Sakaltutan gecidi, Turkey. 1. Epigynum, ventral view. 2. Epigynum, dorsal view. 3. Eyes, view between front and dorsal.

SYSTEMATICS

Family Amaurobiidae Thorell 1870
Subfamily Coelotinae F.O. Pickard-Cambridge 1893
Genus *Coelotes* Blackwall 1841
The *charitonovi* Group

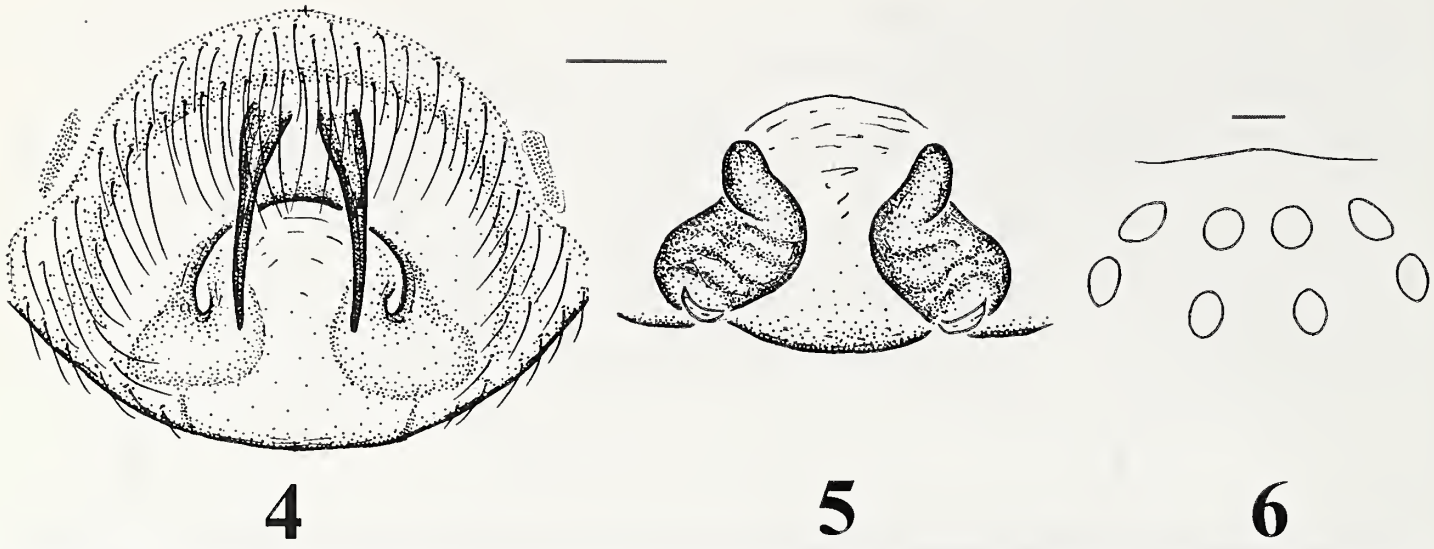
Diagnosis.—Females with anteriorly situated, closely set epigynal teeth; males with strongly modified, broad patellar apophysis, reduced lateral tibial apophysis, short conductor, round, spoon-shaped median apophysis, and prolaterally originating embolus.

Distribution.—Central Asia (Turkey, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan), Near East (Lebanon), Russia (Orenburg Region) (Fig. 37).

Composition.—Eight species: *Coelotes arganoi* Brignoli 1978; *C. caudatus* de Blauwe 1973; *C. charitonovi* Spassky 1939; *C. coenobita* Brignoli 1978; *C. juglandicola* Ovtchinnikov 1984; *C. nenilini* Ovtchinnikov 1999; *C. turkestanicus* Ovtchinnikov 1999; *C. vignai* Brignoli 1978.

KEY TO SPECIES OF THE *CHARITONOVI* GROUP

1. Male (those of *C. caudatus*, *C. arganoi*, *C. coenobita* and *C. vignai* unknown) 2
   Female 5
2. Median apophysis large, distinctly separated from embolic base; embolus thread originating at level of base of tegular sclerite (Figs. 21, 22) ..... *juglandicola*
   Median apophysis small, close to embolic base; embolus thread originating at level near middle of tegular sclerite (Figs. 9, 29) 3
3. Conductor dorsal apophysis length subequal to conductor (Fig. 9) ..... *charitonovi*
   Conductor dorsal apophysis longer than conductor (Fig. 29) 4
4. Conductor dorsal apophysis at least twice the size of conductor (Ovtchinnikov 1999:figs. 41, 42) ..... *nenilini*
   Conductor dorsal apophysis slightly larger than conductor (Fig. 29) ..... *turkestanicus*
5. Atrium situated at level of epigynal hoods (Fig. 26) or anterior of epigynal hoods (Figs. 7, 18) 6
   Atrium situated at level posterior to epigynal hoods (Figs. 1, 4, 15, 34) 9
6. Epigynal teeth contiguous (Fig. 26) 7
   Epigynal teeth distinctly separated (Figs. 7, 18) 8
7. Spermathecae abruptly converging, and then slightly diverging anteriorly (Fig. 27) ..... *turkestanicus*
   Spermathecae gradually converging (Ovtchinnikov 1999:figs. 43, 44) ..... *nenilini*
8. Epigynal teeth with bases separated by at least 3–4 times their width; atrium semi-circular; spermathecal heads extend toward the spermathecal bases (Figs. 18, 19) ..... *juglandicola*
   Epigynal teeth with bases separated by their width; atrium vase-shaped; spermathecal heads extend toward distal spermathecae (Figs. 7, 8) ..... *charitonovi*
9. Atrium with distinct anterior margin; epigynal teeth arising from a transversely extending furrow (Figs. 15, 34) 10
   Atrium without anterior margin; epigynal teeth arising from a smooth surface (Figs. 1, 4) 11
10. Atrium without distinct septum, with anteriorly protruding posterior margin (Figs. 15, 16) ..... *coenobita*
    Atrium with distinct septum (Figs. 34, 35) ..... *vignai*
11. Epigynal teeth relatively short, extend posteriorly less than halfway to epigastric furrow; spermathecae not convergent anteriorly (Figs. 1, 2) ..... *arganoi*
    Epigynal teeth relatively long, extend posteriorly more than halfway to epigastric furrow, spermathecae distinctly convergent anteriorly (Figs. 4, 5) ..... *caudatus*



4 5 6

Figures 4–6.—*Coelotes caudatus* de Blauwe 1973, female holotype from Lebanon. 4. Epigynum, ventral view. 5. Epigynum, dorsal view. 6. Eyes, view between front and dorsal.

Coelotes arganoi Brignoli 1978
Figs. 1–3, 37

Coelotes arganoi Brignoli 1978a:533, Figs. 132, 136 (female holotype, in MHNG, examined).

Material examined.—TURKEY: ♀ holotype, Erzincan, Sakaltutan gecidi, 12 June 1973, R. Argano, L. Boitani & V. Cottarelli (MHNG).

Diagnosis.—Females similar to *C. caudatus* in having anteriorly arising, basally contiguous, distally divergent epigynal teeth, distinct anterior margin of atrium, and broad spermathecae, but can be distinguished by relatively short epigynal teeth that extend less than halfway to epigastric furrow and spermathecae that extend parallel anteriorly (Figs. 1, 2).

Description.—See Brignoli (1978a) for more somatic descriptions.

Female: Medium-sized coelotine, total length 8.65. AME smallest, PME slightly larger than AME, lateral eyes subequal in size, slightly larger than PME; AME separated from each other by slightly less than their diameter, from ALE by about half an AME diameter; PME separated from each other by their diameter, from PLE by almost twice a PME diameter (Fig. 3). Chelicera with 3 promarginal and 3 retromarginal teeth. Epigynal teeth long, slender, arising from slightly anterior to atrium, with contiguous bases and widely separated apices; atrium large, wider than long, situated anteriorly, separated from epigastric furrow by more than its length, with distinct, continuous, arch-shaped anterior margin; copulatory ducts broad, originating anteriorly; spermathecae broad, slightly extended anteriorly, widely separated by about their width; spermathecal heads arising distally on spermathecae (Figs. 1, 2).

Male: Unknown.

Distribution.—Turkey (Fig. 37).

Coelotes caudatus de Blauwe 1973
Figs. 4–6, 37

Coelotes caudatus de Blauwe 1973:31, fig. 29 (female holotype, in MNHN, examined). –Brignoli 1978b:207, fig. 5.

Material examined.—LEBANON: ♀ holotype, Liban, E. Simon (MNHN B 2011, 1.037).

Diagnosis.—Females similar to *C. arganoi* in having the anteriorly arising, basally contiguous, distally divergent epigynal teeth, distinct anterior atrial margin, and broad spermathecae but can be distinguished by relatively long epigynal teeth that extend more than halfway to epigastric furrow and spermathecae that distinctly converge anteriorly (Figs. 4–5).

Description.—Described by de Blauwe (1973) but dorsal view of epigynum was neither illustrated nor described.

Female: Medium-sized coelotine, total length 8.70. Eyes subequal in size, or with ALE slightly larger; AME separated from each other by less than their diameter, from ALE by about AME diameter; PME separated from each other by about 1.5 times their diameter, from PLE by almost twice a PME diameter (Fig. 6). Chelicera with 3 promarginal and 3 retromarginal teeth. Epigynal teeth long, slender, arising anterior of anterior atrial margin, with slightly separated bases and widely separated apices; atrium large, wider than long, situated anteriorly, separated from epigastric furrow by its length, with distinct, continuous, arch-shaped anterior margin; copulatory ducts broad, originating anteriorly; spermathecae broad, with bases separated by their width, slightly extending and converging anteriorly; spermathecal heads arising distally on spermathecae (Figs. 4, 5).

Male: Unknown.

Distribution.—Turkey (Fig. 37).

Coelotes charitonovi Spassky 1939
Figs. 7–14, 37

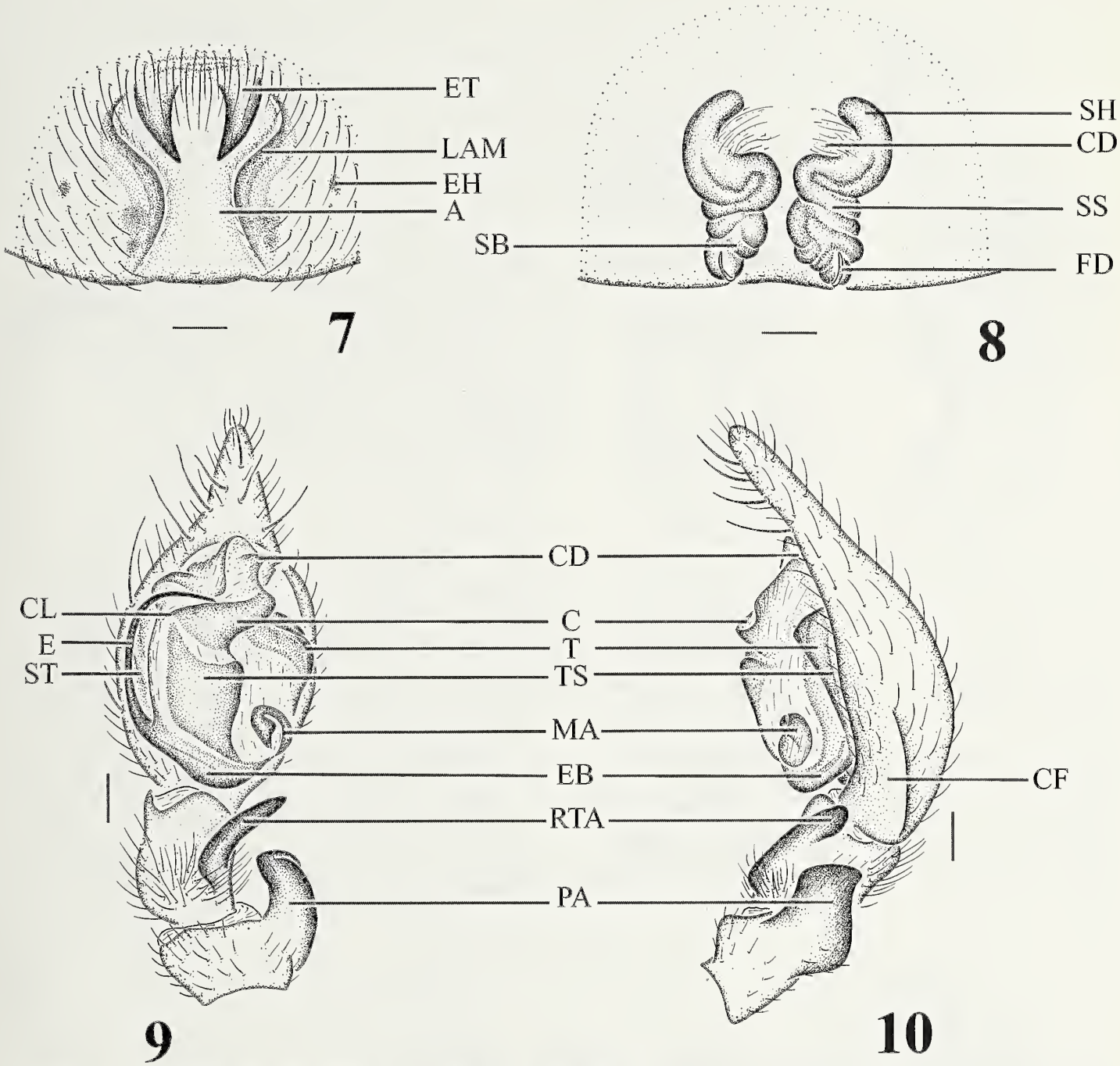
Coelotes charitonovi Spassky 1939:141, fig. 4 (types from Uzbekistan, depository unknown, not examined). –Ovtsharenko and Fet 1980:446; Ovtchinnikov 1999:74, figs. 34, 35; Wang 2002:48, figs. 113–126.

Agelena bucharensis Charitonov 1969:81, fig. 3.

Coelotes bucharensis: Ovtchinnikov 1988:141.

Material examined.—TAJIKISTAN: 1♀1♂, near Khovaling town, 1000 m, 7 November 1987, S. Ovtchinnikov (AMNH-donation of S. Ovtchnnikov).

Diagnosis.—This species is similar to *C. juglandicola* in having the distinctly separated epigynal teeth, but females can



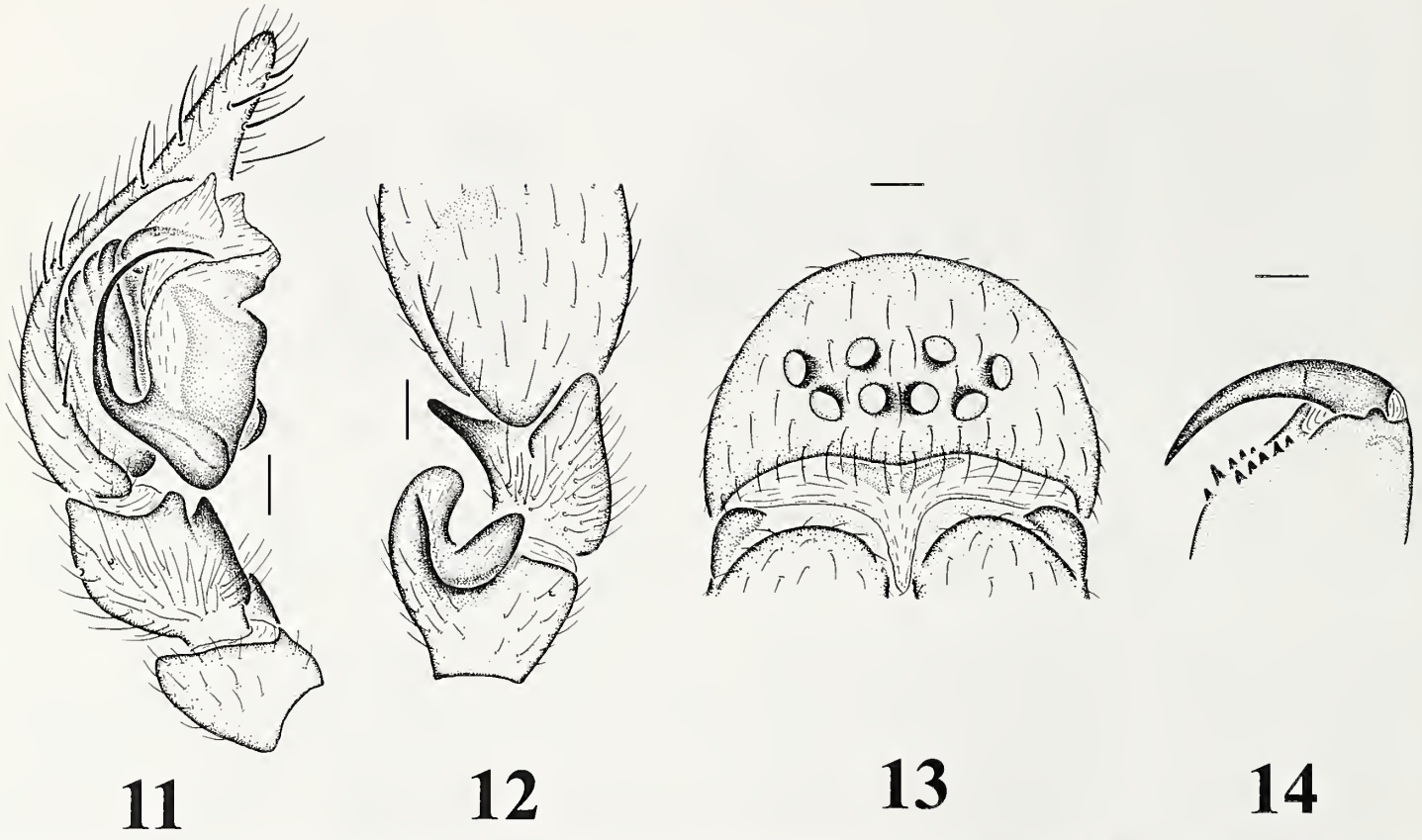
Figures 7–10.—*Coelotes charitonovi* Spassky 1939, female and male from Khovaling town, Tadzhikistan. 7. Epigynum, ventral view. 8. Epigynum, dorsal view. 9. Palp, ventral view. 10. Palp, retrolateral view.

be distinguished by the closely situated epigynal teeth (separated by about their width) and the anteriorly extended spermathecal heads; males by the small conductor (about the size of the conductor dorsal apophysis) and the small, posteriorly situated median apophysis (Figs. 7–12).

Description.—Described by Spassky (1939) but the dorsal view of the epigynum was neither illustrated nor described.

Female: Medium-sized coelotine. ALE largest, other eyes subequal in size, slightly smaller than ALE; AME separated from each other by 2/3 of their diameter, from ALE by about half of AME diameter; posterior eyes equally separated by about 1.5 times PME diameter (Fig. 13). Chelicera with 5 promarginal and 5 retromarginal teeth. Epigynal teeth short, broad, arising from anterior atrial margin, slightly separated by their width; epigynal hoods situated medially; atrium situated anteriorly, with indistinct anterior margin and distinct, vase-shaped lateral margins that extend posteriorly to epigastric furrow; copulatory ducts small, originating anteriorly; spermathecae broad, extend anteriorly, slightly separated; spermathecal heads large, arising distally on spermathecae (Figs. 7, 8).

Male: Medium sized coelotine. Eyes and chelicerae similar to female. Patellal apophysis broad, dorsally concave; RTA more than half the tibial length, with distinctly extending



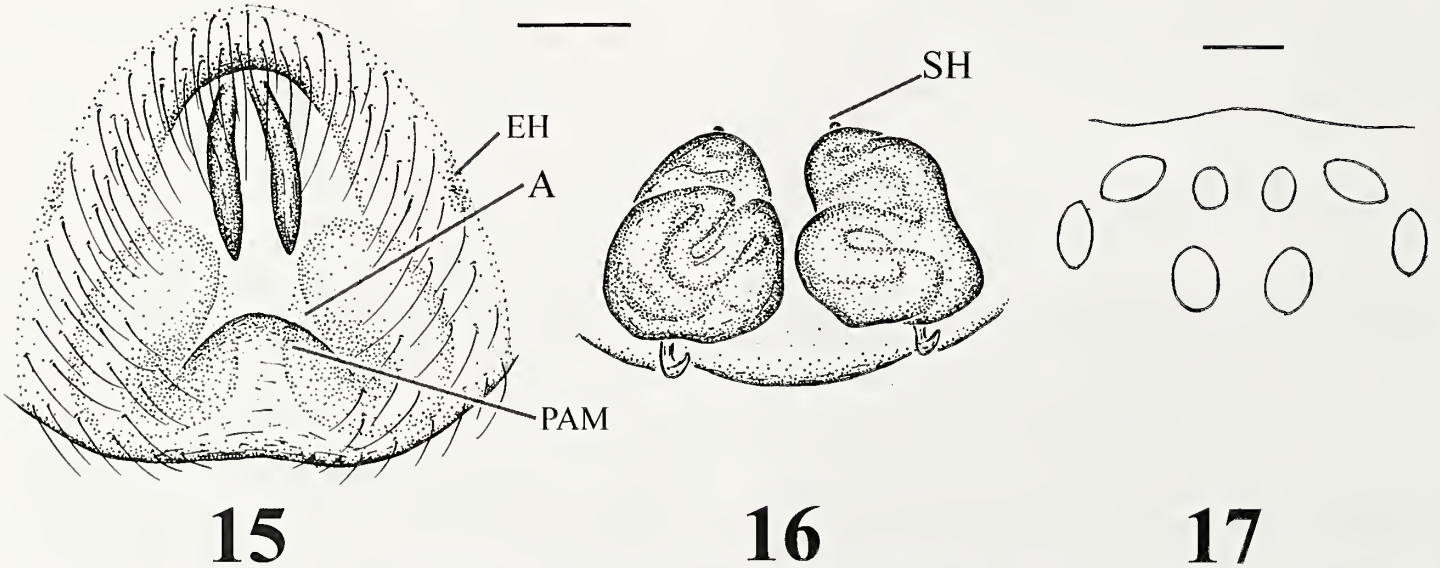
Figures 11–14.—*Coelotes charitonovi* Spassky 1939, female and male from Khovaling town, Tadzhikistan. 11, Palp, prolateral view. 12. Palp, retrolateral view. 13. Female, eyes, front view. 14. Female, chelicera, ventral view.

distal end; lateral tibial apophysis absent; cymbial furrow less than 1/3 of cymbial length; conductor short, basal lamella indistinct, dorsal apophysis about the same size as conductor; median apophysis small, spoon-shaped, without free standing anterior edge, arising posteriorly; embolus short, filiform, prolateral in origin, thread originating near middle of tegular sclerite (Figs. 9–12).

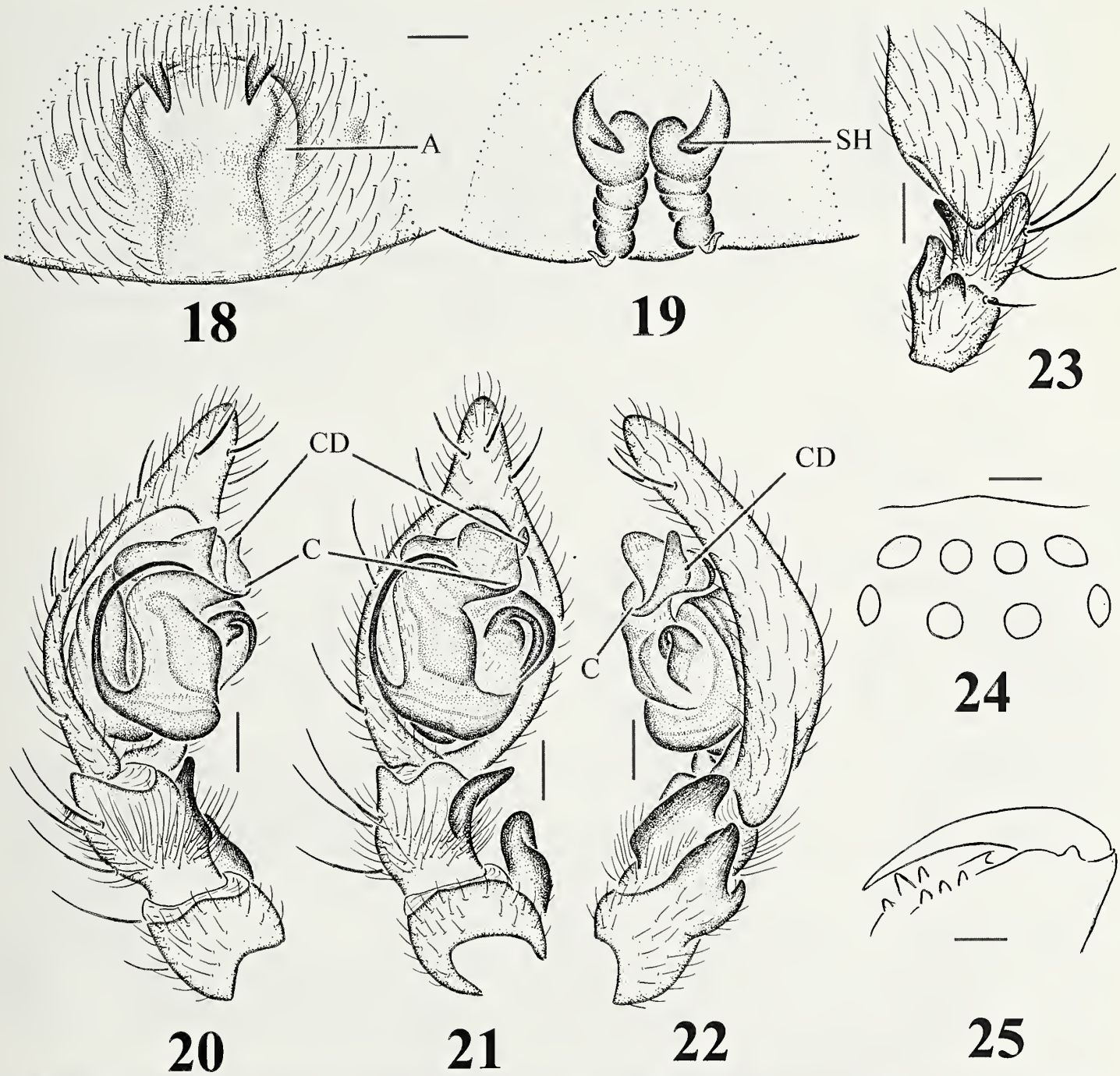
Distribution.—Tajikistan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan (Ovtchinnikov 1999) (Fig. 37).

Coelotes coenobita Brignoli 1978
Figs. 15–17, 37

Coelotes coenobita Brignoli 1978a:533, figs. 135, 137 (female holotype and paratypes, in MHNG, examined).



Figures 15–17.—*Coelotes coenobita* Brignoli 1978, female holotype from Trabzon, Sumela (Macka), Turkey. 15. Epigynum, ventral view; 16. Epigynum, dorsal view; 17. eyes, view between front and dorsal.



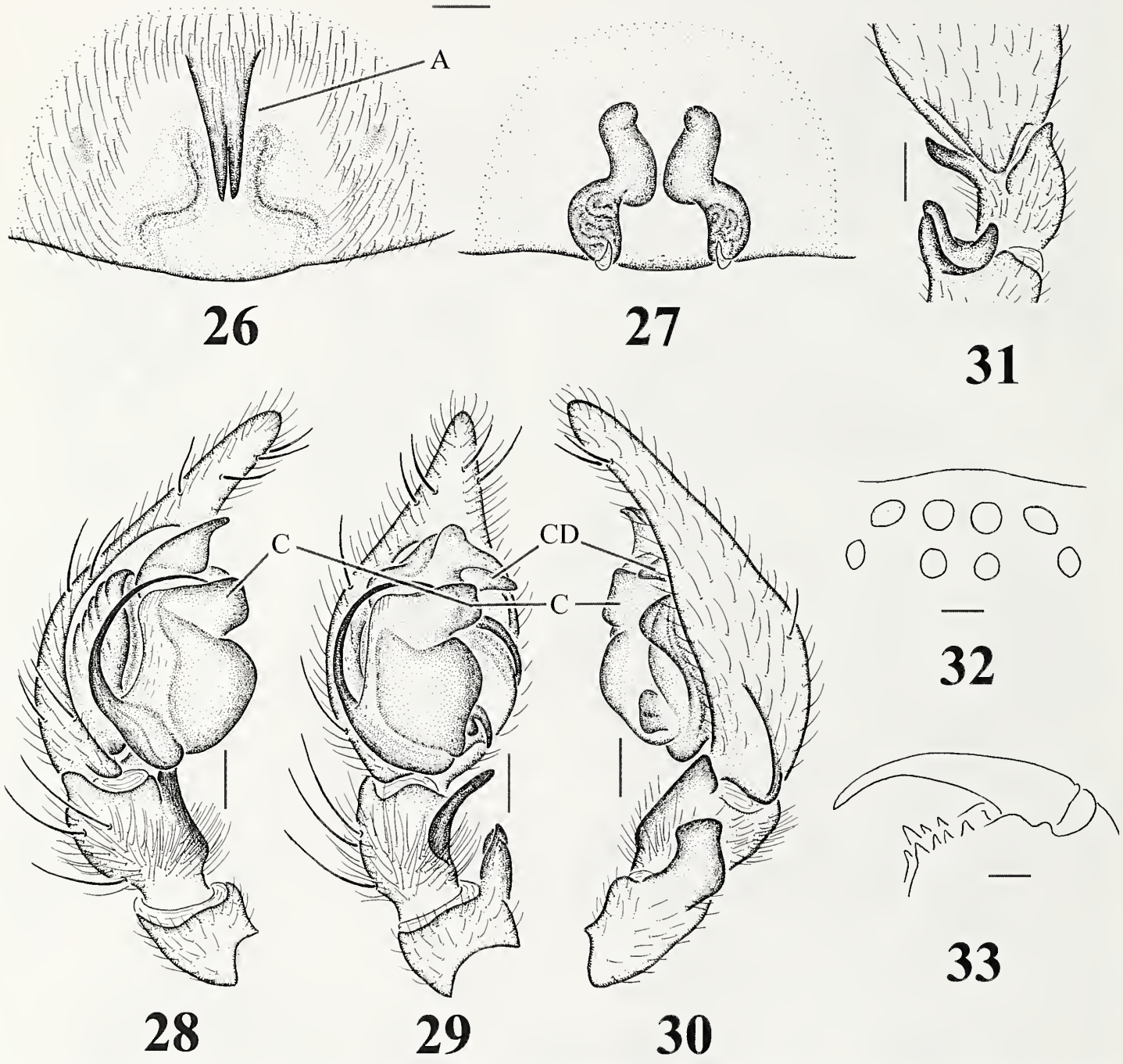
Figures 18–25.—*Coelotes juglandicola* Ovtchinnikov 1984, male and female from Fergansky Mt., Kyrgyzstan. 18. Epigynum, ventral view. 19. Epigynum, dorsal view. 20. Palp, prolateral view. 21. Palp, ventral view. 22, 23. Palp, retrolateral view. 24. Eyes, view between front and dorsal. 25. Chelicera, ventral view.

Material examined.—TURKEY: ♀ holotype, Trabzon, Sumela (Macka), June 16, 1968, P. Brignoli (MHNG); 2♀ paratypes, Trabzon, Sumela (Macka), 10–11 June 1969, P. Brignoli (MHNG).

Diagnosis.—Females are similar to *C. vignai* in having closely arising and extended epigynal teeth, posteriorly situated atrium, and broad, round spermathecae but can be distinguished by the absence of atrial septum, anteriorly extended posterior atrial margin, and slightly separated spermathecae (Figs. 15, 16).

Description.—See Brignoli (1978a) for more somatic descriptions.

Female: Medium-sized coelotine, total length 7.50. AME smallest, about half size of ALE, ALE largest, posterior eyes subequal in size, slightly smaller than ALE; AME separated from each other by about their diameter, from ALE by slightly less than AME diameter; PME separated from each other by less than their diameter, from PLE by more than a PME diameter (Fig. 17). Chelicera with 3 promarginal and 3 retromarginal teeth. Epigynal teeth long, slender, arising anteriorly from a



Figures 26–33.—*Coelotes turkestanicus* Ovtchinnikov 1999, male and female from Kizghizsky Mt., Kyrgyzstan. 26. Epigynum, ventral view. 27. Epigynum, dorsal view. 28. Palp, prolateral view. 29. Palp, ventral view. 30, 31. Palp, retrolateral view. 32. Eyes, view between front and dorsal; 33. Chelicera, ventral view.

transversely extending furrow, close together; atrium situated posteriorly, with distinct, anteriorly convexing posterior margin; copulatory ducts indistinct from dorsal view; spermathecae broad, round, close together; spermathecal heads arising distally on spermathecae, barely visible (Figs. 15, 16).

Male: Unknown.

Distribution.—Turkey (Fig. 37).

Coelotes juglandicola Ovtchinnikov 1984
Figs. 18–25, 37

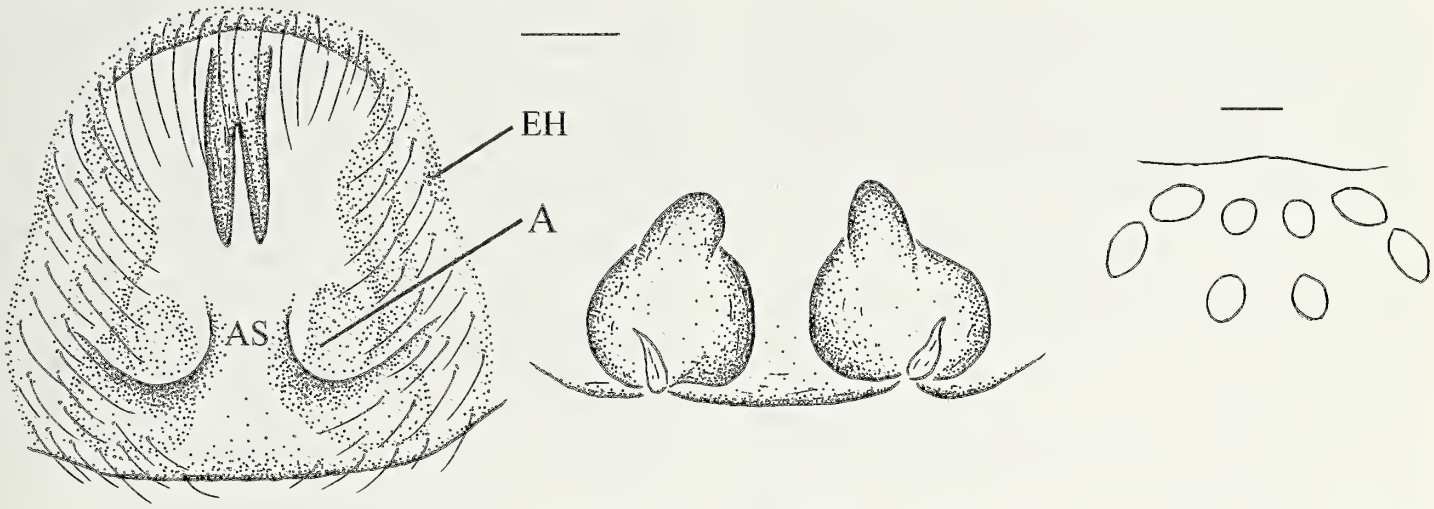
Coelotes juglandicola Ovtchinnikov 1984:126, figs. 1–2 (types not examined).

Material examined.—KYRGYZSTAN: 1♂1♀, Fergansky Mt., Baubashata Mts., Arslanbop, 17 July 1991, S. Ovtchinnikov (AMNH-donation of S. Ovtchinnikov).

Diagnosis.—Males can be recognized by embolus with thread originating near base of tegular sclerite, and females by widely separated epigynal teeth and posteriorly extended spermathecal heads (Figs. 18–23).

Description.—Described by Ovtchinnikov (1984) but the dorsal view of epigynum was neither illustrated nor described.

Female: Medium-sized coelotine. ALE largest, slightly larger than other eyes, which are subequal; anterior eyes equally separated by slightly more than half of AME diameter;



Figures 34–36.—*Coelotes vignai* Brignoli, 1978, female holotype from Bolu, Abant, Turkey. 34. Epigynum, ventral view. 35. Epigynum, dorsal view. 36. Eyes, view between front and dorsal.

PEM separated from each other by their diameter, from PLE by slightly less than 1.5 times PME diameter (Fig. 24). Chelicerae with 3 promarginal and 3 retromarginal teeth (Fig. 25). Epigynal teeth short, arising from anterior atrial margin, widely separated by about three times their width; epigynal hoods situated medially; atrium situated anteriorly, with continuous, arch-shaped anterior margin; copulatory ducts small, originating anteriorly; spermathecae broad, extending and converging anteriorly, separated by their width at bases and contiguous at apices; spermathecal heads small, arising distally and extending toward spermathecal bases (Figs. 18, 19).

Male: Medium-sized coelotine. Eyes and chelicerae similar to female. Patellar apophysis broad, dorsally concave; RTA more than half length of tibia, with distinctly extended distal end; lateral tibial apophysis absent; cymbial furrow less than

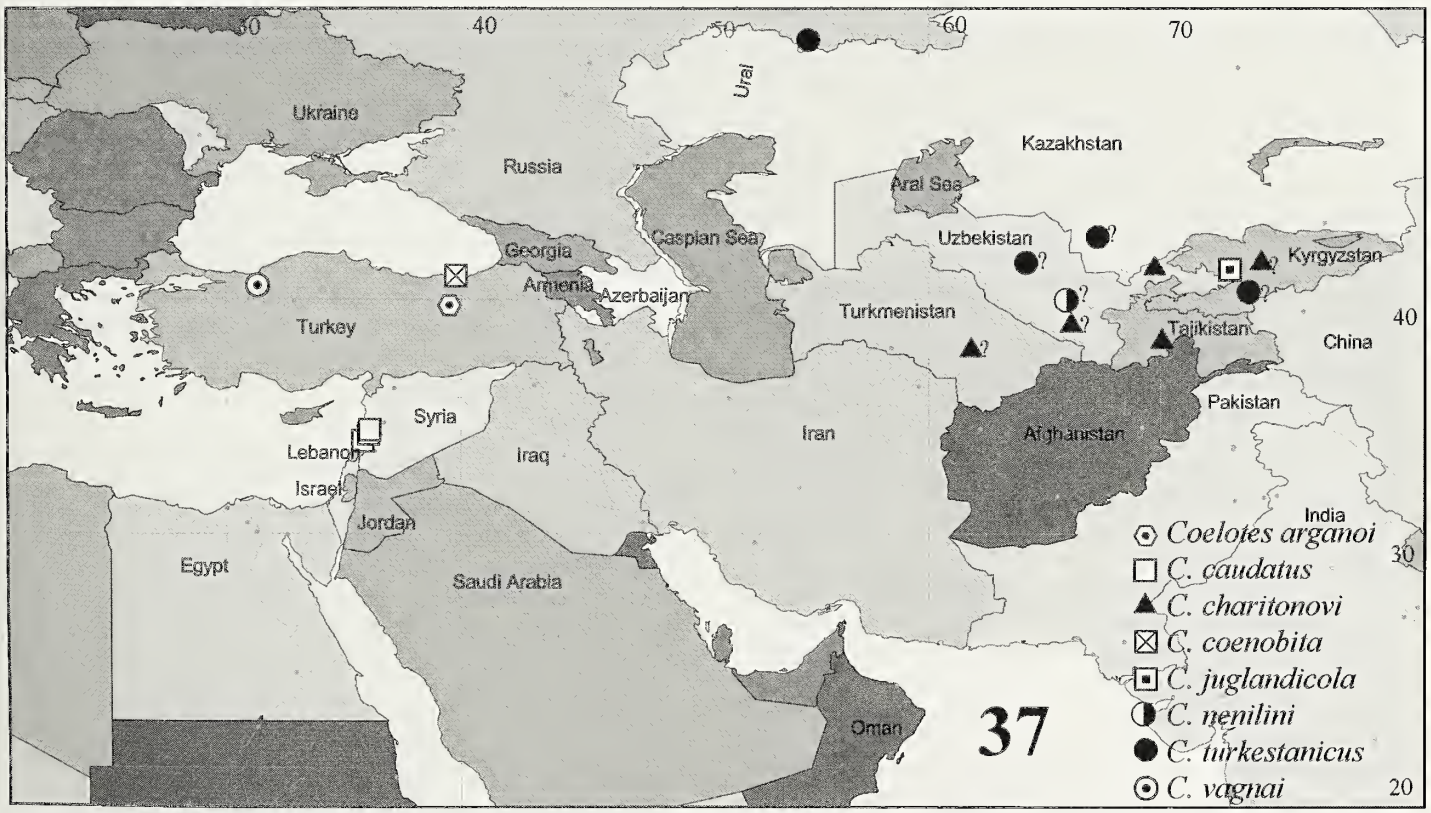


Figure 37.—Records of *charitonovi* group species (a question mark behind the symbol indicates the presence in the corresponding country, but without detailed locality).

1/3 of cymbial length; conductor short, basal lamella indistinct, dorsal apophysis about as large as conductor; median apophysis relatively large, spoon-shaped, without free standing anterior edge, distinctly separated from embolic base; embolus short, filiform, prolateral in origin, thread originating near base of tegular sclerite (Figs. 20–23).

Distribution.—Kyrgyzstan (Fig. 37).

Coelotes nenilini Ovtchinnikov 1999
Fig. 37

Coelotes nenilini Ovtchinnikov 1999:77, figs. 40–44 (types not examined).

Material examined.—None.

Diagnosis.—Similar to *C. turkestanicus* in having the long, contiguous epigynal teeth and the elongated spermathecae, but can be distinguished by the gradually convergent spermathecae in females and the large conductor dorsal apophysis in males.

Description.—Based on the illustrations of Ovtchinnikov (1999:figs. 41–44).

Female: Epigynal teeth long, contiguous, arising anteriorly; epigynal hoods situated medially; atrium reduced to slit; copulatory ducts indistinct; spermathecae extended and slightly convergent anteriorly, separated by about their width; spermathecal heads large, arising distally on spermathecae.

Male: Patellar apophysis broad, dorsally concave; RTA more than half length of tibia, with distinctly extended distal end; lateral tibial apophysis absent; cymbial furrow about 1/4 of cymbial length; conductor short, basal lamella indistinct, dorsal apophysis much larger than conductor; median apophysis small, spoon-shaped, without free standing anterior edge, situated close to embolic base; embolus short, filiform, prolateral in origin, thread originating near middle of tegular sclerite.

Distribution.—Uzbekistan (Ugamsky Mt. Range) (Fig. 37).

Coelotes turkestanicus Ovtchnnikov 1999
Figs. 26–33, 37

Coelotes turkestanicus Ovtchinnikov 1999:75, figs. 36–39 (male and female paratypes, in AMNH, examined). –Esyunin, Tuneva & Farzalieva 2007:53, figs. 14–16, 25.

Material examined.—KYRGYZSTAN: 1♂2♀ paratypes, Kizghizsky Mt. R., Malinovka Canyon, 1700 m, 21 October 1984, S. Ovtchinnikov (AMNH, donation of S. Ovtchnnikov).

Diagnosis.—Similar to *C. nenilini* in having the long, contiguous epigynal teeth and elongated spermathecae, but can be distinguished by abruptly convergent spermathecae in female and male conductor dorsal apophysis slightly larger than conductor (Figs. 26–31).

Description.—See Ovtchinnikov (1999) for more somatic descriptions.

Female: Large-sized coelotine, total length 12.60. Anterior eyes subequal in size or with ALE slightly larger, posterior eyes subequal, slightly smaller than anterior eyes; anterior eyes separated by about half an AME; PME separated from each other by about their diameter, from PLE by twice a PME diameter (Fig. 32). Chelicerae with 4 promarginal and 4 retromarginal teeth (Fig. 33). Epigynal teeth long, contiguous, arising anteriorly; epigynal hoods situated medially; atrium reduced to slit; copulatory ducts indistinct; spermathecae extending and abruptly converging anteriorly, separated by more than their width at bases and slightly separated at apices; spermathecal heads large, arising distally on spermathecae (Figs. 26, 27).

Male: Large-sized coelotine, total length 10.00. Eyes and chelicerae same as in female. Patellar apophysis broad, dorsally concave; RTA more than half length of tibia, with distinctly extended distal end; lateral tibial appphysis absent; cymbial furrow about 1/4 of cymbial length; conductor short, basal lamella indistinct, dorsal apophysis slightly larger than conductor; median apophysis small, spoon-shaped, without free standing anterior edge, situated posteriorly, close to embolic base; embolus short, filiform, prolateral in origin, thread originating near middle of tegular sclerite (Figs. 28–31).

Distribution.—Kazakhstan, Kyrgyzstan, Uzbekistan, Russia (Fig. 37).

Coelotes vignai Brignoli 1978
Figs. 34–37

Coelotes vignai Brignoli, 1978a:535, fig. 133 (female holotype and paratypes, in MHNG, examined).

Material examined.—TURKEY: 1♀ holotype, 3♀ paratypes, Bolu, Abant, 12 July 1971, A. Vigna (MHNG); 2♀ paratypes, Bolu, Abant, 1400 m, 17 July 1971, P. Brignoli (Coll. Brignoli).

Diagnosis.—Females similar to *C. coenobita* in having closely arising and extended epigynal teeth, posteriorly situated atrium, and the broad, round spermathecae but can be distinguished by the presence of atrial septum and separated spermathecae (Figs. 34, 35).

Description.—See Brignoli (1978a) for more somatic descriptions.

Female: Medium-sized coelotine, total length 9.31. AME smallest, about half the size of ALE, ALE largest, PLE slightly smaller than ALE, PME slightly larger than AME; AME separated from each other by less than their diameter, from ALE by about half of AME diameter; PME separated from each other by about their diameter, from PLE by about 1.5–2 times a PME diameter (Fig. 36). Chelicera with 3 promarginal and 3 retromarginal teeth. Epigynal teeth long, slender, arising anteriorly, close together; atria situated posteriorly, with distinct septum; copulatory ducts indistinct from dorsal view; spermathecae broad, round, slightly separated; spermathecal heads large, arising distally on spermathecae (Figs. 34, 35).

Male: Unknown.

Distribution.—Turkey (Fig. 37).

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Predation by *Misumenops pallidus* (Araneae: Thomisidae) on insect pests of soybean cultures in Buenos Aires Province, Argentina

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Abstract. This study analyzes predation by adult females of *Misumenops pallidus* (Keyserling 1880) on pairs of prey items representing non-pest insects and potential pests. The phenology of the potential pests was such that each insect guild peaked sequentially, while non-pest herbivorous and insectivorous insects were present during the entire period. Field experiments were made in a commercial 50-ha soybean plot during two successive years. Ten cages 1 × 1 × 0.5 m were placed in peripheral furrows of a soybean commercial plot. The pest species were preyed on differentially, with the order from the most favored species to the least with respect to non-pest herbivorous and insectivorous insects was as follows: defoliating lepidopterous larvae, seed feeding pentatomids in their early nymphal instars, stem boring lepidopterous larvae, and seed feeding pentatomids in older nymphal and adult instars. Adult females of *M. pallidus* fed on all the insect species offered, but in the presence of defoliator larvae, they hardly accepted alternative prey, whereas in the presence of other prey, they maintained a more generalized diet.

Keywords: Agroecosystems, biological control, natural enemies

True predators kill their prey more or less immediately after attacking them, and during their lifetime they will kill several or many different prey individuals. Although most true predators have relatively broad diets, some degree of preference is almost always present (Begon et al. 1996). There is evidence that generalist arthropod predators choose to eat certain prey to balance their amino-acid requirements and therefore may be affected by previous feeding (Greenstone 1979).

While most ecological studies on spiders as potential biocontrol agents in agroecosystems have focused on Lycosidae, Linyphiidae, and Araenidae, much less is known about Thomisidae (Dean et al. 1987; Agnew & Smith 1989; Lang et al. 1999; Symondson et al. 2002; Vichitbandha & Wise 2002; Romero & Vasconcello- Neto 2003; Harwood et al. 2004). In soybean plots in the rolling pampa (Argentina), thomisids represented 47.2% of all spiders collected in the herbaceous stratum, and *Misumenops pallidus* (Keyserling 1880) is the most abundant species (Liljesthröm et al. 2002). It is a rather small (<10 mm) spider that hunts preferentially in the upper and medium strata of the soybean plants. Following the classification proposed by Uetz et al. (1999), it belongs to the guild of hunting ambushers. *M. pallidus* has a relatively long developmental period, and presumably only one generation occurs during the soybean growth period. However, *M. pallidus* exhibits some characteristics of "agrobiont" spiders (Luczak 1979). As a species, it disperses widely, colonizes crops from the beginning of their development, and the adults (particularly females) are found throughout the entire soybean cycle. Apart from this, daily predation rates increase with increasing age of the spider, so that the total number of prey killed by an adult female represents 81% of prey consumed during its lifetime (Liljesthröm et al. 2002; González et al. 2009).

The most important examples of potential insect prey in soybean fields are represented by three guilds (Root 1967) of primary or secondary pests: intermediate-sized defoliating lepidopterous larvae, stem boring lepidopterous larvae, and seed feeding pentatomids, as well as a complex of other non-pest herbivorous and insectivorous insects (Bimboni 1985; La Porta & Crouzel 1985; Gamundi 1985; Aragón & Belloso 1987; Bercellini & Malacalza 1994; Luna & Sánchez 1999b). Previous results showed that in the laboratory *M. pallidus* preyed on all prey types except curculionids and adult stink bugs (Cheli et al. 2006). In the present study area, the defoliating guild was represented by *Rachiplusia nu* Guennée (Lepidoptera: Noctuidae) (Luna & Sánchez 1999a), the stem-boring guild by *Crocidosema* (=*Epinotia*) *aporema* (Walsingham) (Lepidoptera: Tortricidae) (Luna et al. 1996; Liljesthröm & Rojas 2005), and the seed-feeding stink bugs by *Nezara viridula* (Linnaeus) and *Piezodorus guildinii* West. (Hemiptera: Pentatomidae) as the most abundant species (Bimboni 1985; Bercellini & Malacalza 1994; Liljesthröm & Coviella 1999; Frana 2008). Among non-pest herbivorous and insectivorous insects, the following were the most abundant: *Colaspis* sp. and *Diabrotica speciosa* (Germ.) (Coleoptera: Chrysomelidae), *Eriopis conexa* (Germ.), *Cycloneda sanguinea* (Germ.), *Coccinella* sp. (Coleoptera: Coccinelidae), unidentified species of Membracidae and Cicadellidae (Homoptera: Auchenorrhynca), and a species of Curculionidae (Aragón & Belloso 1987; Bercellini & Malacalza 1994).

Although non-pest insects were recorded during the entire soybean growth period with only small variations in density, the phenology of the potential pests is such that each guild peaks sequentially. *R. nu* larvae peaked during the early vegetative stages of soybean growth (V4–V6) in late January during two consecutive years, mean peak density was 17.6 larvae/ linear meter; stem-boring larvae peaked in numbers (9.1 individuals/ linear meter) during later vegetative stages (V7–V8) in mid February. The small instars of seed feeders peaked during reproductive stages (R3–R6) in mid March (mean peak density was 11.2 larvae/ linear meter) and large

instars peaked 2–3 wk later at a lower density (Aragón & Belloso 1987; Bercellini & Malacalza 1994; González et al. in press).

In this work, we analyze daily predation by adult female *M. pallidus* on pairs of different prey types, each pair consisting of one pest and one non-pest species. We hypothesized that *M. pallidus* would prey indistinctly on all prey except adult curculionids (a component of non-pest insects in some treatments) and adult stink bugs, and that they would exhibit similar preferences toward all pairs of prey types offered, excluding the former.

METHODS

Predation by adult female *M. pallidus* was estimated in four field experiments conducted in a commercial 50-ha soybean plot located in Chivilcoy (34°54′S, 60°02′W), Buenos Aires province, during two successive years. The area belongs to the pampean phytogeographic province (Cabrera 1976). The climate is humid-mesothermal, and apart from cattle, the main agricultural products are soybean, maize, and wheat.

Two kinds of potential prey (a pest and a non-pest species) were offered in each of four experiments that were repeated in both years, and the density of potential prey (4 per cage) represented approximately the mean pest density (expressed as the number of individuals per linear meter of crop) during the entire phenological soybean period in the field (González et al. 2009). In each experiment, we used 10 cages, 1 × 1 × 0.5 m, covered with a nylon net (1 × 1 mm). The cages were placed in peripheral furrows of a commercial soybean plot. The area where each cage would be placed was first cleared by hand. All litter, soybean plants, weeds, and insects were eliminated, and the bare ground flattened and compacted. Then we placed three plastic pots containing soybean plants in a phenological state similar to those of the crop and covered them with the cage (which was sunk into the soil to prevent the entrance of ground-active arthropods). We placed potential prey individuals in each cage, as follows:

1) Two *R. nu* (3^{rd}–4^{th} larval instars; defoliators) and two non-pest insects (*D. speciosa* and *Coccinella* sp. in the first year, and *D. speciosa* and *Colaspis* sp. in the second year). The experiment was carried out in late January when defoliating lepidopterous larvae peaked.
2) Two *C. aporema* (4^{th}–5^{th} larval instars, which were placed on a leaf near a bud. It was expected that each larva would protect itself by stitching together silk threads or boring a stem before a spider and two non-pest insects (*D. speciosa* and a membracid in the first year and *D. speciosa* and an unidentified curculionid in the second year) were released into the cage. The experiment was carried out in mid-February when stem-boring lepidopterous larvae peaked.
3) Two *N. viridula* (3^{rd} instar nymphs, seed-feeding) and two non-pest insects (*D. speciosa* and a membracid in the first year and only *D. speciosa* in the second year). The experiment was carried out in mid-March when seed-feeding pentatomid nymphal instars peaked.
4) Two *N. viridula* (5^{th} instar nymphs) and adult *P. guildinii* (both seed-feeders) and two non-pest insects (*Coccinella* sp., which preyed mainly on aphids and Thysanoptera, and *D. speciosa* in the first year and only *D. speciosa* in the second year). The experiment was carried out in late March, when adult stink bugs usually exhibit maximum density.

The prey species and adult spiders used in the experiments were collected in the field with a 40-cm diameter sweeping net. The specimens were deposited in the Arachnological Laboratory of the Center of Parasitological Studies (University of La Plata) at the study's conclusion. The potential prey were placed in the 10 cages (grouped in pairs of a pest and a non-pest species) at the beginning of the experiments and left for 24 hours to allow acclimation. Adult female spiders were collected in the field three to four days before, and after a 48-hour fasting period were provided with an adult *Drosophila melanogaster* for standardization of hunger level. Then one spider was introduced into each of five cages selected at random, while the remaining five cages were spider-free controls. Prey and predators were kept together for 24 hours; then we disassembled the cages and counted the number of individuals remaining.

For each of the four combinations of prey items and for both years, we analyzed predation by adult female *M. pallidus* by calculating the predation rate per cage (Kajak et al. 1968). We calculated the daily predation rate per cage on the i-th prey type, DPR(i), as:

$$\mathrm{DPR}(i) = [\mathrm{No}(i) - \mathrm{Nf}(i) - \mathrm{Ns}(i)]/\mathrm{No}(i)/\Delta t$$

where No(i) and Nf(i) represent, respectively, the initial and final number of the i-th prey type in the cage with spiders, Ns(i) the mean number of missing i-th prey type in the control cages, and $\Delta t = 1$ day, the duration of the experiment. Each year, possible differences between treatments were analyzed by MANOVA, and in each treatment DPR(i) values corresponding to the *i-th* insect pest and non-pest insects in each experiment was examined with a t-test. We also calculated the total number of all prey types preyed on per cage per day:

$$\mathrm{DPR} = [\mathrm{No}(i) - \mathrm{Nf}(i) - \mathrm{Ns}(i)] + [\mathrm{No}(NPI) - \mathrm{Nf}(NPI) - \mathrm{Ns}(NPI)]$$

Differences among treatments in both years (prey killed per cage) were tested by ANOVA, and homogeneity of variances was tested by Levene's test. No transformation was necessary.

RESULTS

We recorded the disappearance of only one insect from the control cages: a *R. nu* in the second year. In the treatment cages *M. pallidus* caught prey of all those offered except the adult curculionid and *P. guildinii*. However, the predation rates showed that some pests were preferred over non-pest insects, while others were less preferable. The same tendency was observed both years: $F_{3,\ 30} = 7.154$; $P = 0.00008$ (first year), and $F_{3,\ 30} = 3.997$; $P = 0.0047$ (second year) (Figs. 1a, b).

When we analyzed each treatment separately, the defoliating lepidopterous larvae exhibited a higher predation rate than

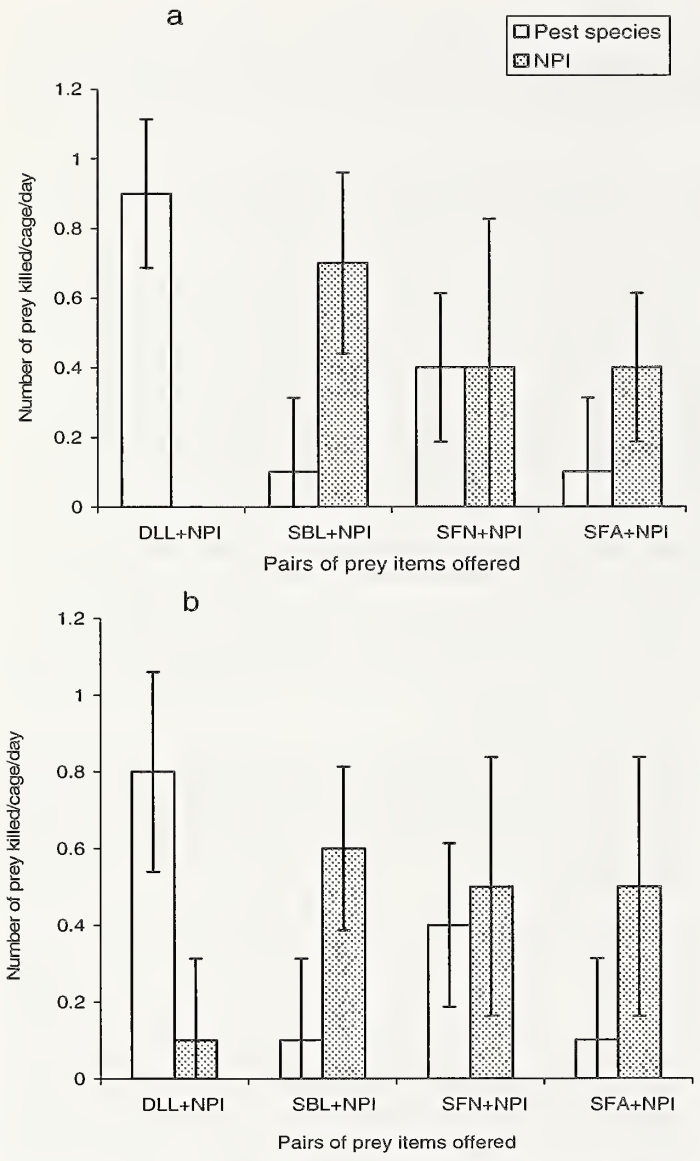

Figure 1a, b.—Predation rate of an adult female *Misumenops pallidus* when a pest species and non-pest insects were offered. a. During the first year of studies; b. During the second year of studies. (DLL = defoliator lepidopterous larvae, SBL = stem borer lepidopterous larvae, SFN = seed feeding pentatomids in the first three nymphal instars, SFA = seed feeding pentatomids in older nymphal instars and in the adult stage NPI = non-pest herbivorous and insectivorous insects). Confidence intervals at 90% probability.

non-pest insects. Conversely, in both years the stem-boring lepidopterous larvae were preyed on less than the non-pest insects. In both years the predation rate on stink bugs did not differ statistically from that of non-pest insects. In the first three nymphal instars, the predation rate on the bugs was similar to that of non-pest insects. When bugs were later instar nymphs or in the adult stage, the predation rate on later-stage nymphs and adults was lower than that on non-pest insects, but not significantly so. In the latter case, only late-instar nymphs, but not adults, were preyed on.

For the total number of prey killed daily per cage, the daily predation rates varied from 1 to 1.8 in the first year, but differences were not significant ($F_{3,16} = 1.85$, $P < 0.18$). In the second year, minimum and maximum daily predation rates were 1.2 and 1.8, respectively, and non-significant ($F_{3,16} = 1.02$, $P < 0.41$). Thus, regardless of the prey types offered, the spiders killed approximately the same number of prey items.

DISCUSSION

Adult female *M. pallidus* killed one to almost two prey items per cage per day and fed on all the offered insect species except adult stink bugs and adult curculionids. These values are congruent with field estimates by Edgar (1969) and Nyffeler & Benz (1987, 1988); however, prey densities could be different in those situations. *M. pallidus* acted almost as a specialist predator in the presence of the defoliating *R. nu*, whereas in the presence of other prey guilds, it showed a more varied feeding pattern. Yet the low attack rate of the spider on the stem s is likely due to a low encounter rate with this prey guild, similar to small defoliating larvae. The stem-boring larvae remain hidden from potential predators inside cocoons made of leaves stitched together with silk threads. However, they are parasitized by larval parasitoids (Liljesthröm & Rojas 2005). Other species of herbivores that spend a large proportion of their life cycles in cryptic locations are not susceptible to significant predation by carabids, staphylinids, and spiders but are susceptible to specialist natural enemies (Rämert & Ekbom 1996). On the other hand, the low attack rate on stink bugs is likely due to a certain degree of invulnerability of this prey guild, particularly adults because of their relatively large size, their thick cuticle, and production of toxic compounds (Aldrich 1988; Staddon 1979; Pareja et al. 2007). These factors probably explain why most of the predation on this guild was on fifth-instar nymphs (mainly by *N. viridula*, which is larger than *P. guildinii*); we only saw nymphs preyed on by adult spiders in the field. The thickness of the adult curculionid cuticles could also account for our results.

Studies by Turnbull (1960) and Riechert & Lockley (1984) demonstrated prey size selectivity in spiders, and Nentwig (1983) showed that most of his web-building spiders caught and ate almost anything small enough for them to handle that arrived in their webs, although they rejected prey that was toxic or whose cuticule was too hard to penetrate.

Spiders are the main natural enemies of pest insects in some agroecosystems, which makes them agronomically significant (Riechert & Lockley 1984). Notwithstanding, early biological control studies focused on specialist predators rather than on spiders because, as generalists, they were thought to have relatively little impact on agricultural pests (Savory 1928; Bristowe 1941; Comstock 1965). This view has changed, and different studies have demonstrated the capacity of spiders to reduce the population density of some pest insects and their consequent damage (Riechert & Lockley 1984; Nyffeler et al. 1992; Riechert & Lawrence 1997; Marshall et al. 2002). Still, other studies indicate limited pest control potential under certain conditions (Holland & Thomas 1997; Lang et al. 1999; Birkhofer et al. 2008). On the other hand, since effective biological control is the result of complex interactions at the community level, the activity of spiders would be complemented by that of other natural enemies (Sunderland 1999), although it was observed in the field that *M. pallidus* preyed on the taquinid *Trichopoda giacomellii* (Blanchard), a parasitoid of *N. viridula* (Liljesthröm, pers. obs.), and coccinelid predators (this study).

Polyphagy helps to sustain predators when pest density is low in agroecosystems, usually early in the season. Generalists may already be present, subsisting on non-pest prey, while specialist parasitoids may take some time to build up in numbers (Symondson et al. 2002). In soybean cultures in the study area, defoliating and stem-boring larvae have different guilds of parasitoids (Luna & Sánchez 1999a; Liljesthröm & Rojas 2005), while late-instar nymphs and adults of *N. viridula* are attacked by *T. giacomellii* (Liljesthröm & Bernstein 1990; Liljesthröm & Rabinovich 2004). Yet the only arthropod natural enemies found on young-instar nymphs of *N. viridula*, as well as on adults and immature stages of *P. guildinii*, are predators, among which spiders are the most abundant (Bercellini & Malacalza 1994; Liljesthröm et al. 2002). We found many other spider species representing seven guilds that reached relatively high densities (Liljesthröm et al. 2002) and could prey on certain potential pest species. Even though their effect is unknown in the agroecosystem, they are important mortality factors on the pest species considered in this study, as well as on other phytophagous insects that rarely attain pest status.

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Karyotypes of the Neotropical pseudoscorpions *Semeiochernes armiger* and *Cordylochernes scorpioides* (Pseudoscorpiones: Chernetidae)

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Abstract. The karyotypes and course of meiosis of two pseudoscorpions, *Semeiochernes armiger* (Balzan 1892) and *Cordylochernes scorpioides* (Linnaeus 1758) (Pseudoscorpiones: Chernetidae), are described for the first time. The diploid chromosome number of the male is 69 in *S. armiger* and 47 in *C. scorpioides*. As in most pseudoscorpions studied to date, autosomes exhibit predominantly biarmed morphology. Both species possess an X0 sex chromosome system. In most pseudoscorpions with X0 system karyotyped so far, including European chernetids, the X chromosome exhibits metacentric morphology. In contrast, the X chromosome of both neotropical chernetids studied exhibits asymmetric, submetacentric morphology.

Keywords: Pseudoscorpiones, Chernetidae, karyotype, sex chromosome, X0 sex chromosome determination

With more than 3,380 described species, Pseudoscorpiones is the fourth largest order of the arthropod class Arachnida (Harvey 2008). Despite this considerable species diversity, the morphology of pseudoscorpions is very conservative, and it can therefore be difficult to distinguish between closely related species on the basis of morphological features alone (Zeh & Zeh 1994; Wilcox et al. 1997; Zeh et al. 2003). Moreover, in many groups of pseudoscorpions, species identification is also complicated by the lack of detailed analysis of intraspecific morphological variability. For example, researchers originally described the neotropical pseudoscorpion, *Semeiochernes armiger* (Balzan 1892), from Central and South America as three species (*S. armiger*, *S. extraordinarius*, and *S. militaris*) on the basis of sexually dimorphic traits of the male pedipalpal chelae (Beier 1933, 1954). However, rearing experiments have demonstrated that intrapopulation variability encompasses the full range of *Semeiochernes* "interspecific" chelal morphology and that species status cannot be established using these male characters (Zeh & Zeh 1992).

Recent studies suggest that molecular and cytogenetic characters in pseudoscorpions diverge more rapidly than morphological traits and may thus prove particularly useful for identifying cryptic species and for resolving fine-scale evolutionary relationships. For example, the harlequin beetle riding pseudoscorpion, *Cordylochernes scorpioides* (Linnaeus 1758), ranging from Costa Rica to southern Brazil, was described by Beier (1948) as a single species, based on morphological examination of hundreds of specimens from several countries in South and Central America. However, mitochondrial cytochrome oxidase I (COI) gene sequencing has revealed extensive genetic differentiation, with a maximum likelihood nucleotide divergence of nearly 33% between *C. scorpioides* populations from Panama and northern South America (Trinidad and French Guiana) (Zeh et al. 2003). This extreme molecular divergence is associated with complete postzygotic incompatibility between individuals from central Panama and both French Guiana (Zeh & Zeh 1994) and Trinidad (J.A. Zeh, unpublished data), indicating that geographic populations of *C. scorpioides* constitute a complex of cryptic species. Interestingly, researchers have documented extensive mitochondrial COI sequence divergence between individuals from Panama and Trinidad in *S. armiger*, suggesting that the pattern exhibited by *C. scorpioides* may be common and that many neotropical pseudoscorpion species may actually represent cryptic species complexes (Wilcox et al. 1997).

Karyotype data also holds great promise as a tool for differentiating between closely related taxa. There is considerable karyotype diversity in all genera of pseudoscorpions that have been studied in detail to date, namely *Roncus* (Neobisiidae) (Troiano 1990, 1997), *Chthonius* (Chthoniidae) (Šťáhlavský & Král 2004), *Lasiochernes* (Chernetidae) (Šťáhlavský et al. 2005), *Geogarypus* (Geogarypidae), and *Olpium* (Olpiidae) (Šťáhlavský et al. 2006). In this paper, we present the results of the first cytogenetic study of *S. armiger* and *C. scorpioides*. Our karyotype analyses of these two neotropical representatives of the family Chernetidae not only contribute valuable data for comparing patterns of karyotype evolution in neotropical and European chernetid pseudoscorpions, but also provide a basis for future investigation of the relationship between karyotype evolution, molecular divergence, and speciation in *Semeiochernes* and *Cordylochernes*.

METHODS

All pseudoscorpions used in this study were derived from populations inhabiting decaying fig trees (*Ficus* spp.) in the lowland rain forest of the former Canal Zone, Republic of Panama (9°N, 79°W). Voucher specimens of *C. scorpioides* and *S. armiger* have been deposited with W.B. Muchmore (University of Rochester, USA), V. Mahnert (Muséum d'histoire naturelle, Geneva, Switzerland), and D. Quintero (Universidad de Panamá, Republic of Panama).

Semeiochernes armiger (Balzan 1892): 6 males collected in January 2006 either as adults (n = 3) or as tritonymphs that molted to the adult stage in the laboratory (n = 3).

Cordylochernes scorpioides (Linnaeus 1758): 8 males and 8 females from a large laboratory population established from 35 females collected in the field in August 2000.

Chromosome preparations were made using the technique described by Šťáhlavský & Král (2004). Briefly, gonads were dissected, hypotonised in 0.075 M KCl for 15 min, and fixed in a mixture of methanol:glacial acetic acid (3:1) for at least 20 min. We placed a piece of fixed material into a drop of 60% acetic acid on a clean microscope slide suspended by a pair of tungsten needles. Then we

Figure 1.—*Semeiochernes armiger*, male karyotype. Based on two sister metaphase II plates. Bar = 5 μm.

transferred the slide onto a warm histological plate (surface temperature of 40–45° C) and moved the drop of dispersed tissue on the slide with a tungsten needle until it evaporated. The chromosome preparations were air-dried at room temperature overnight and stained with 5% Giemsa solution in Sörensen phosphate buffer (pH = 6.8) for 40 min.

Chromosome morphology was classified according to Levan et al. (1964). We calculated relative chromosome length as a percentage of the total length of the diploid set, including the sex chromosome. Owing to the small number of suitable spermatogonial mitotic metaphase plates, we used sister metaphase II for analysis of karyotypes in males. In addition, the centromere positions are much more obvious in pseudoscorpions at this meiotic stage.

RESULTS

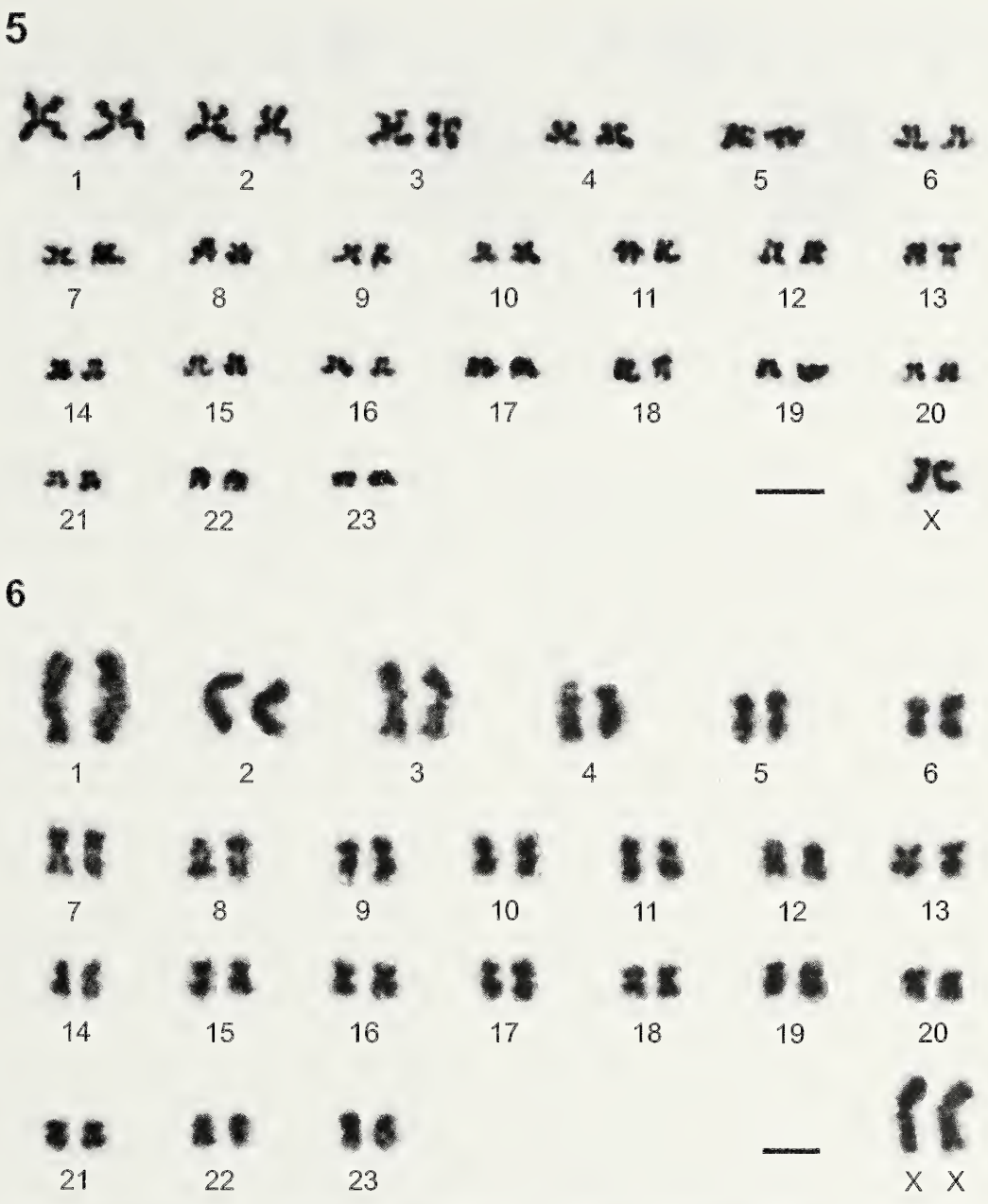
Semeiochernes armiger (Balzan 1892)

The male diploid complement comprises 69 chromosomes. The karyotype contains 18 pairs of metacentric (Nos. 4, 5, 8, 9, 11, 12, 14, 15, 16, 18, 19, 20, 21, 22, 25, 27, 30, 31), seven pairs of submetacentric (Nos. 1, 2, 7, 10, 13, 28, 29), three pairs of subtelocentric (Nos. 3, 17, 24), and six pairs of acrocentric (Nos. 6, 23, 26, 32, 33, 34) autosomes (Fig. 1). The first three pairs of autosomes are slightly larger than the other pairs (Fig. 1), and their relative size decreases from 3.4% to 2.5% of the diploid set. The remaining autosomes decrease gradually in size from 1.9% to 0.7% of the diploid set.

The sex chromosome system is X0. The X chromosome shows submetacentric morphology (centromeric index 1.83), constitutes 2.1% of the diploid set, and exhibits more intensive staining than other chromosomes (i.e., positive heteropycnosis) during some periods of meiotic division. During meiosis (Figs. 2–4), more intensive staining revealed overcondensation of the X chromosome from leptotene to pachytene (Fig. 2) and during metaphase II (Fig. 4). By contrast, we noted that all chromosomes are isopycnotic during metaphase I (Fig. 3).

Chiasma frequency is relatively low. In diplotene - metaphase I plates (n = 19), we observed at least one bivalent with two chiasmata and maximally four bivalents with two chiasmata. The mean chiasma frequency was 1.08 per bivalent.

Figures 2–4.—Course of meiosis in *Semeiochernes armiger*. 2. Pachytene. 3. Metaphase I. 4. Metaphase II cell containing X chromosome. Arrowheads indicate the X chromosome, arrow points to the bivalents with two chiasmata. Bars = 10 μm.



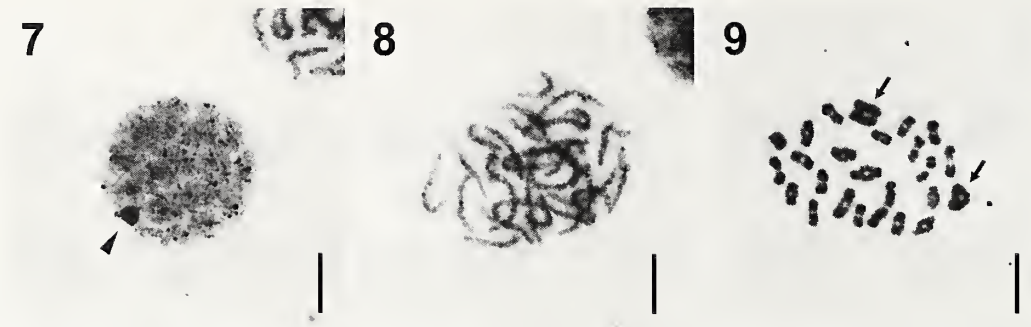
Figures 5–6.—*Cordylochernes scorpioides*. 5. Karyotype of male. Based on two sister metaphase II plates. 6. Karyotype of female. Based on mitotic metaphase. Bars = 5 μm.

Cordylochernes scorpioides (Linnaeus 1758)

The diploid number is 47 in males (Fig. 5) and 48 in females (Fig. 6). The karyogram of the species is based on two sister metaphases II of a male (Fig. 5). The karyotype is composed of 17 metacentric (Nos. 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 13, 14, 16, 17, 18, 20, 21), three submetacentric (Nos. 8, 12, 15), and three subtelocentric (Nos. 19, 22, 23) pairs of autosomes (Fig. 5). The relative length of the autosomes decreases gradually from 4.4% to 1.4% of the diploid set in male metaphase II and from 4.8% to 1.2% of the diploid set in female mitotic metaphase. Comparison of the male and female karyotypes, as well as analysis of male meiosis, revealed an X0 sex chromosome system. The X chromosome is submetacentric (centromeric index 1.75) and relatively large, forming 3.6% of the diploid set in males and 2.7% in females. As in *S. armiger*, the X chromosome of *C. scorpioides* exhibits positive heteropycnosis in germinal cells of males. However, we detected heteropycnosis only during premeiotic interphase (Fig. 7). During late pachytene (Fig. 8), metaphase I (Fig. 9), and metaphase II (Fig. 5), the X chromosome appeared to be isopycnotic with the autosomes. During male meiosis, *C. scorpioides* exhibited lower chiasma frequency than *S. armiger* (number of analyzed diplotene plates = 378). We calculated the mean chiasma frequency as 1.03 per bivalent. More specifically, we observed two bivalents with two chiasmata in five diplotene nuclei and one bivalent with three chiasmata in five diplotene nuclei. In the remaining cells, all bivalents had only one chiasma.

DISCUSSION

Until now, karyotype descriptions of the family Chernetidae have been limited to five species, all from the European region. Sokolow (1926) published basic data on the karyotype of *Dendrochernes cyrneus* (L. Koch 1873) in his pioneering analysis of spermatogenesis in pseudoscorpions. However, nearly 80 years elapsed before



Figures 7–9.—Course of meiosis in *Cordylochernes scorpioides*. 7. Premeiotic interphase. 8. Late pachytene. 9. Metaphase I. Arrowhead indicates the X chromosome, arrows point to the bivalents with two chiasmata. Bars = 10 μm.

scientists conducted any further karyotype analyses of chernetid species. In a study aimed at reconstructing the phylogeny of arthropod telomeric sequences, Vítková et al. (2005) provided only data on the diploid number and telomeric sequences of *Chernes hahnii* (C.L. Koch 1839). Šťáhlavský et al. (2005) provided the first detailed descriptions of chernetid karyotypes, which were limited to three species in the genus *Lasiochernes*. Despite limited comparative data, European chernetids appear to be characterized by high diploid chromosome number (49–73), a predominance of biarmed chromosomes, and the presence of an X0 sex chromosome system (Šťáhlavský et al. 2005).

In this study, we present the first karyotype descriptions of pseudoscorpions from the Neotropics. As with previously karyotyped pseudoscorpions (e.g., Troiano 1997; Šťáhlavský & Král 2004; Šťáhlavský et al. 2005, 2006), neotropical chernetids exhibit considerable karyotype variability. The diploid chromosome number of the *C. scorpioides* male (2n = 47) is the lowest known diploid number within chernetids. By contrast, the male karyotype of *S. armiger* consists of 69 chromosomes. Despite this disparity in chromosome number, the karyotypes of both neotropical species have several features in common with European chernetids. Their karyotypes are characterized by high 2n, as well as by a predominance of biarmed chromosomes. As with the majority of pseudoscorpions karyotyped so far, all representatives of the family Chernetidae possess an X0 sex chromosome system. Remarkably, European and neotropical chernetids differ in the morphology and relative size of the sex chromosome. In all karyotyped European species, the sex chromosome is metacentric and is the largest element of the karyotype (Šťáhlavský et al. 2005). Metacentric morphology of the X chromosome has been found in the majority of pseudoscorpions exhibiting the X0 sex chromosome system (Troiano 1990, 1997; Šťáhlavský & Král 2004; Šťáhlavský et al. 2005, 2006). Interestingly, in both neotropical chernetids, the X chromosome exhibits submetacentric morphology. Moreover, it is not the largest element of the karyotype. These fundamental differences may be the result of long-term isolation and divergent evolution of the X chromosome in European and neotropical chernetids. Clearly, many additional cytogenetic and molecular systematic studies of species and populations from both biogeographical regions are needed in order to gain a better understanding of karyotype evolution and diversity in chernetid pseudoscorpions.

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Palpal urticating hairs in the tarantula *Ephebopus*: fine structure and mechanism of release

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Abstract. The tarantula genus *Ephebopus* is exceptional with respect to its urticating hairs: they are located on the palps rather than on the abdomen, as is the rule for other Neotropical tarantulas. These urticating hairs occupy a small field of 1–2 mm^2 on the medial side of the palpal femora. Each urticating hair measures 500–600 μm in length and 5–6 μm in diameter. Almost the entire hair shaft is studded with little barbs that point toward the hair tip. Urticating hairs arise from a slipper-shaped socket in the cuticle, at an angle of 25–30°. When the spider is threatened, it shows a brief palpal flick as a defensive reaction, whereby many urticating hairs are brushed off and fly through the air. These hairs do not have a preformed breaking point but become detached at the very base and are then pulled out from their sockets, like an arrow from a quiver. The actual release behavior occurs too quickly (0.1 s) to be followed by the naked eye. Video film analyses reveal that a single upward movement of the palps rubbing against the lateral surfaces of the spread chelicerae causes the dispersal of urticating hairs into the air.

Keywords: Morphology, defensive behavior, Neotropical tarantulas

Many Neotropical tarantulas defend themselves by brushing off special urticating hairs from their bodies (Bates 1863; Bertani & Marques 1995/96). When these hairs come in contact with the skin, eyes, or respiratory tract of a threatening animal, they can cause serious irritations (Cooke et al. 1972) or allergic reactions (Castro et al. 1995). Commonly, urticating hairs are located on the abdomen and are brushed off with the hind legs. However, there is one exception in the genus *Ephebopus*, a colorful South American tarantula, where the urticating hairs occur on the palps rather than on the abdomen (Raven 1985). These hairs form distinct fields, so-called "pedipalpal brushes" on the medial surfaces of the femora; the hairs are released into the air by a flick of the palps. Marshall & Uetz (1990) provided a first description of the morphology of the urticating hairs in *Ephebobus*, their release after provocation, and their effect on laboratory mice. However, several points remained unclear: 1) structure, size, and total number of urticating hairs, 2) attachment and detachment of these hairs from their sockets, and 3) details about the actual release mechanism during the defensive reaction. We addressed the morphological questions using light and scanning electronmicroscopical techniques. For analyzing which body parts were involved in releasing the urticating hairs, we performed frame-by-frame analysis on several video recordings of defensive reactions.

METHODS

Exuvia of subadult *Ephebopus cyanognathus* West & Marshall 2000 were used for all microscopical studies. Isolated urticating hairs were inspected and measured under the light microscope. Entire fields of urticating hairs were excised and embedded in Epon; 1–2 μm sections were stained with methylene blue and examined in a phase contrast microscope. For scanning electron microscopy palpal brushes were sputtered with gold and viewed from various directions in a Zeiss DSM 950. Digital photographs were taken at magnifications from 20× to 5000×. For a more three-dimensional representation of the attachment sites, we cut several palpal brushes into longitudinal strips with a razor blade and then mounted them sideways before examination in the scanning electron microscope. For comparative purposes we also studied nearby sensory hairs.

In order to observe the defensive reaction, a transparent glass vial was moved toward the spider from in front. Slightly touching the front legs often triggered a palpal flick and a concomitant release of urticating hairs. Since this happened very fast, we used a video camera (Sony DCR PC107E) with 25–30 pictures/s and frame-by-frame analysis. Overall we elicited about 20 defensive reactions and captured three of them on video film.

All six *Ephebopus cyanognathus* spiders used in this study were bred in captivity by one of the authors (BR). They were 18 mo old, subadult, and still about two molts away from maturity. With a body length of 3 cm they were slightly smaller than the adults.

RESULTS

The palpal brushes are located on the medial sides of the femora, rather distally and near the patellar joint. They occupy a patch of 1–2 mm^2 that is easily visible with the naked eye (Fig. 1). The term "pedipalpal brush" is quite descriptive since the hairs are tightly packed and run parallel to each other, like a paint brush (Fig. 2). In contrast to the surrounding sensory hairs, which are dark brown or black, and the ornamental hairs, which are deep blue, the urticating hairs have a golden-reddish color. Their orientation is almost parallel to the axis of the femur, but slightly deflected ventrally. A single urticating hair is 500–600 μm long but only 5–6 μm thick. The ratio of length/width is thus around 100:1, giving the hair a needle-like appearance. As is typical for urticating hairs, the hair shaft bears numerous pointed barbs, each about 5 μm long (Figs. 2, 5). They are lacking at the very base, but appear as small cuticular extensions, just after the hair comes out of its socket (Fig. 8). The barbs are spaced rather regularly at an interval of 10 μm along the entire hair shaft, which also bears fine longitudinal ridges. The distal end of the urticating hair is acutely pointed, suggesting that it represents the penetrating tip. However, since all the barbs are pointing distally, this would quickly prevent any further penetration into the skin (see Discussion).

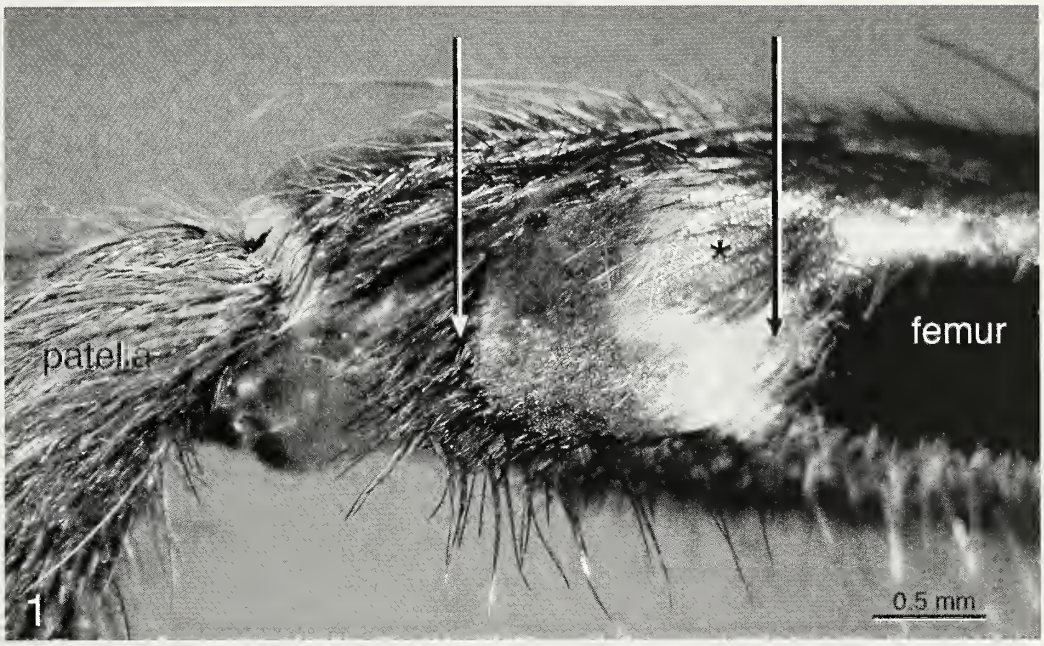

Figure 1.—Medial surface of the femur of *Ephebopus cyanognathus*, near the patella joint. The two arrows mark the location of the "pedipalpal brush," a region that consists of thousands of urticating hairs. A small area (asterisk) is devoid of such hairs, because they were brushed out during a defensive reaction.

At the proximal end the hair shaft becomes very smooth and decreases in diameter from 5 to 2–3 μm. It also exhibits a slight bend where it disappears into the socket (Figs. 4, 8). The angle at which the hair shaft emerges from the socket lies between 25 and 30°, which means that the urticating hairs are lying rather flat on the surface of the femur.

The sockets are slipper-shaped, about 10 μm long and 5–6 μm wide, rising 3–4 μm above the leg cuticle (Figs. 3, 4, 7). The

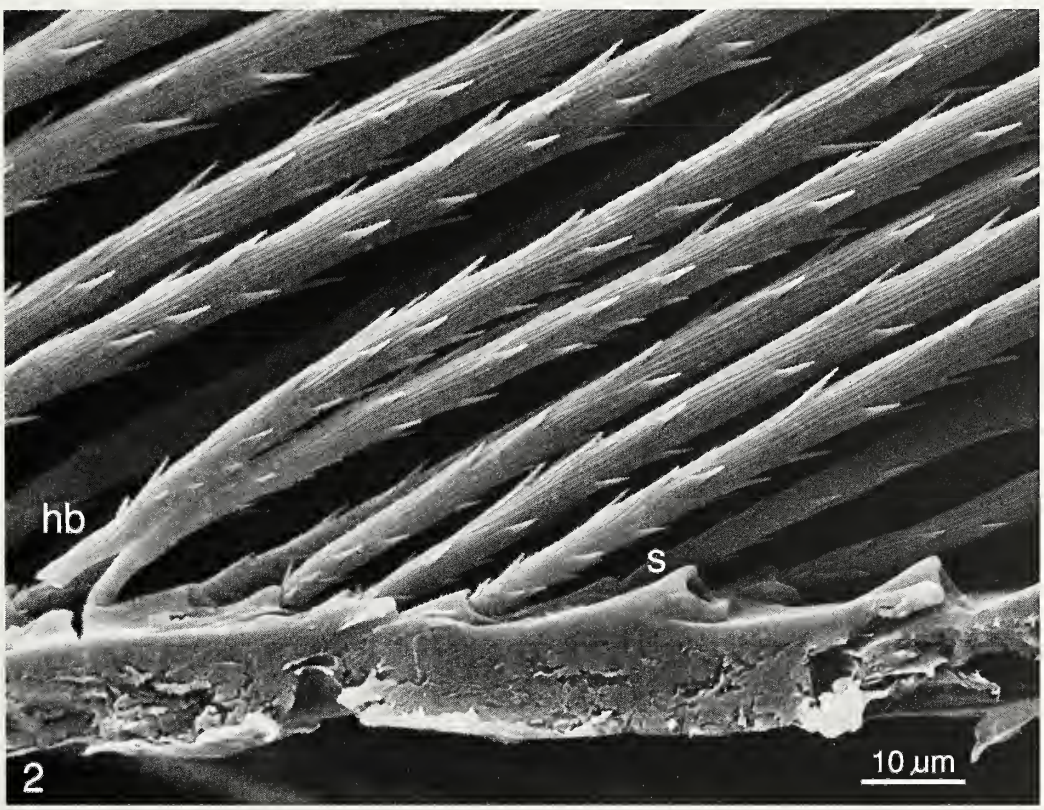

Figure 2.—The pedipalpal brush cut longitudinally: the barbed urticating hairs are seen from the side. Each hair arises from a slipper-shaped socket (s) at an angle of 25–30°. Some urticating hairs have become detached from their sockets, and their free hair base (hb) is visible on the left.

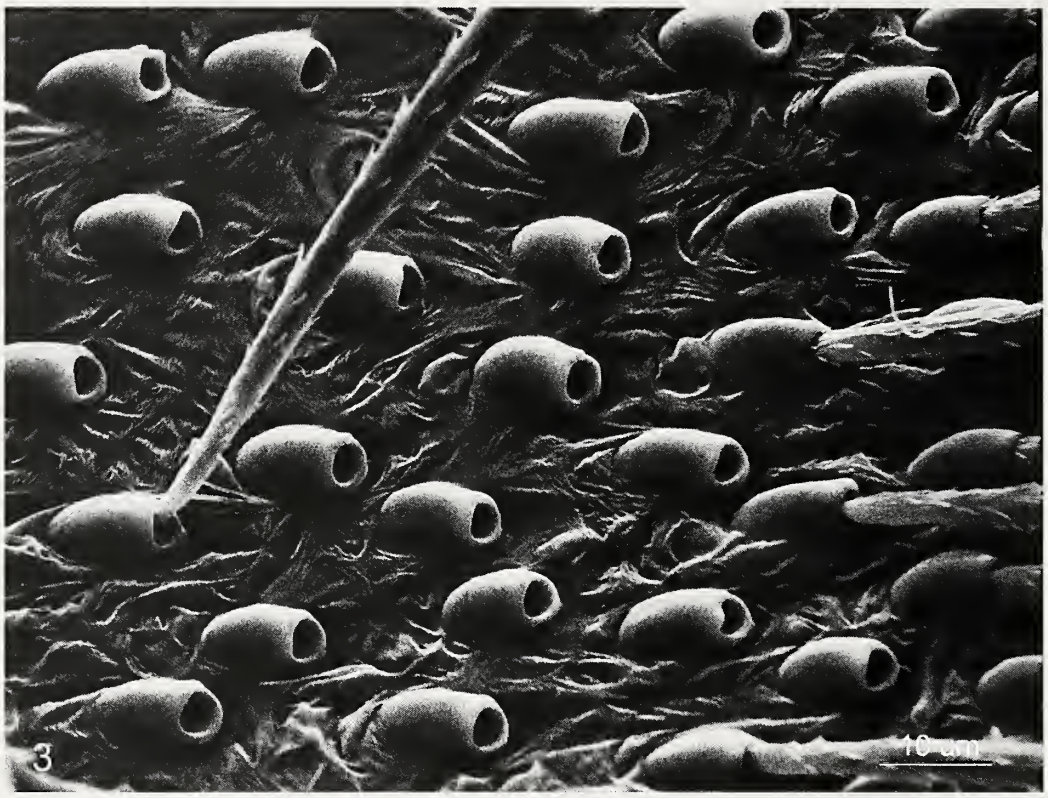

Figure 3.—A marginal region of the pedipalpal brush showing mostly empty sockets, because the urticating hairs had been brushed off in a defensive reaction. A few hairs (on the right) are still held within their sockets, but the single hair on the left has been pulled out partially. Note that there is no breaking point at the hair base.

opening for the hair shaft measures 3–4 µm, allowing a bit more movement vertically than horizontally. The basal part of the hair shaft fits snugly into its socket and ends at a basal ring (Figs. 9, 10). Below that hollow, circular structure a canal of about 5 µm in diameter traverses the cuticle vertically (Figs. 4, 7, 9).

How is the urticating hair attached to its socket? It appears that only the most proximal rim of the hair base is connected to the basal ring, via a thin cuticular membrane. This membrane can be seen in its original position in Fig. 9, and remnants are often found attached to isolated hairs at the very

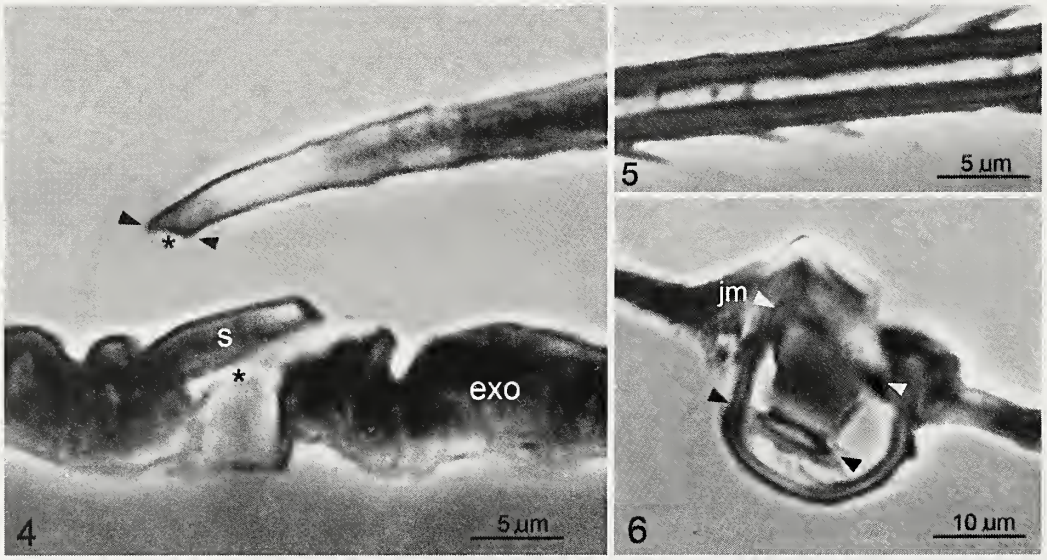

Figure 4.—Longitudinal section of a socket (s) and the detached base (arrowheads) of an urticating hair. The two asterisks mark the original position of the hair base inside the socket. The exocuticle (exo) of an exuvium is only 5–10 µm thick.

Figure 5.—For most of its length, the hair shaft is barbed on the outside and hollow inside. The central lumen is only 1–2 µm wide and exhibits small struts crossing the lumen.

Figure 6.—Section of the base of a large sensory hair showing fine membranous connections to the socket (black arrowheads). A much stronger joint membrane (jm) is responsible for the firm attachment of the hair shaft to the socket (white arrowheads).

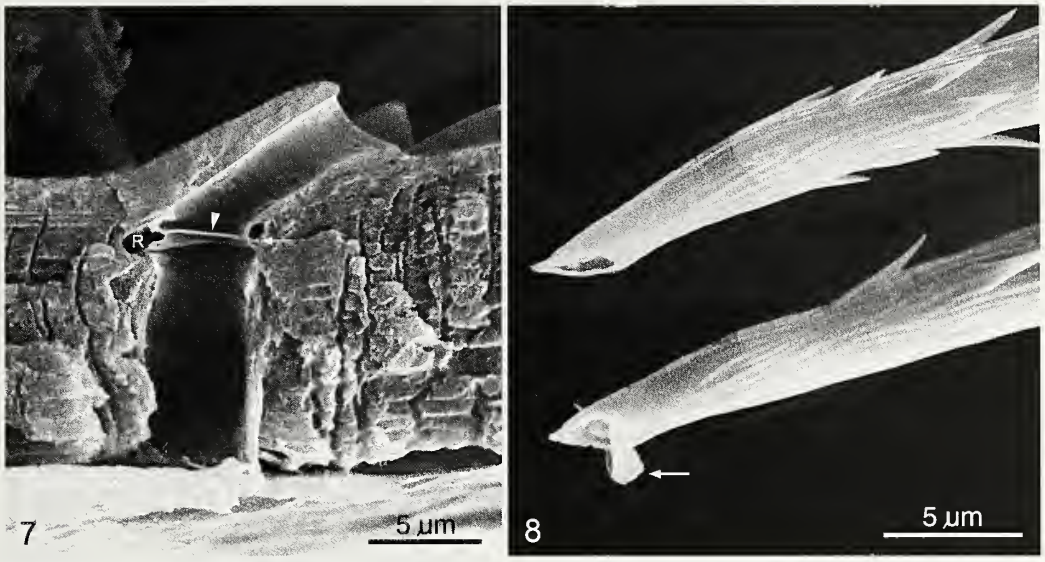

Figure 7.—A sagittal section of an empty socket of an urticating hair. The oblique upper part of the canal, which normally houses the base of the hair shaft, ends in a horizontally running membrane (arrowhead). That is the spot where the hair base is attached laterally to a ring-like structure (R); it appears as two small circles, because the ring is seen in cross-section (arrow). Further below, the canal continues in a vertical direction through the exocuticle.

Figure 8.—Two hair bases of detached urticating hairs. Note the obliquely pointed endings reminiscent of a hypodermic needle. The part of the hair shaft that is normally concealed within the socket has been shaded. Fragments of the attachment membrane (arrow) often still adhere to the hair base.

base (Fig. 8). The hair shaft seems to be hollow and thus very fragile at the attachment site; it quickly becomes more solid distally, with a wall thickness of 2 µm and a central lumen of 1 µm (Fig. 5). The delicate connection via a membrane is probably the key factor when the hair becomes dislodged from its socket. A corresponding membrane connecting the hair base to the socket is also present in the large sensory hairs (Fig. 6). However, sensory hairs have an additional and much stronger membrane, the joint membrane, that anchors the hair shaft movably yet firmly in the socket.

It is noteworthy that we never found broken urticating hairs. Any released urticating hair (detached either naturally by the spider or artificially with a needle) remains in one piece. We found no stumps of broken hair shafts inside the socket. An exception is pictured in Fig. 9, but there the hair shaft had been crushed by the razor blade when we cut the cuticle.

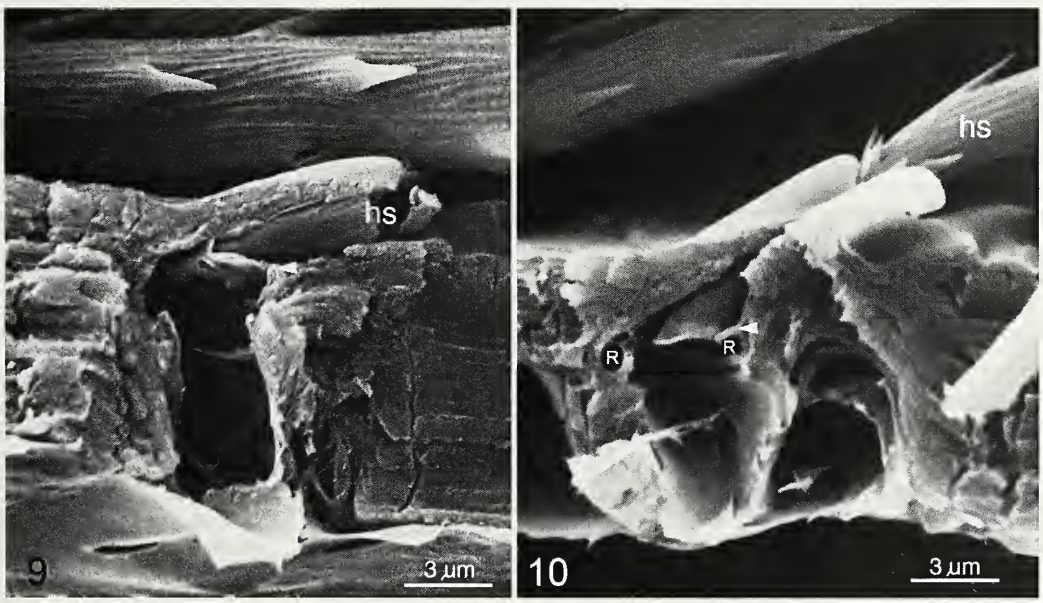

Figure 9.—Parasagittal section of the attachment of an urticating hair. In this case the hair shaft (hs) had been broken inadvertently when the cuticle was cut with a razor blade. However, the attachment membrane (arrowhead) at the base of the socket is clearly visible. Note the narrow diameter of the hair shaft inside the socket (2–3 µm), compared to a more distal portion (top of figure; 5–6 µm).

Figure 10.—A similar parasagittal section, but with the hair shaft (hs) still intact. The arrowhead marks the ending of the hair base and the surrounding ring-like structure (R; *cf.* Fig. 7).

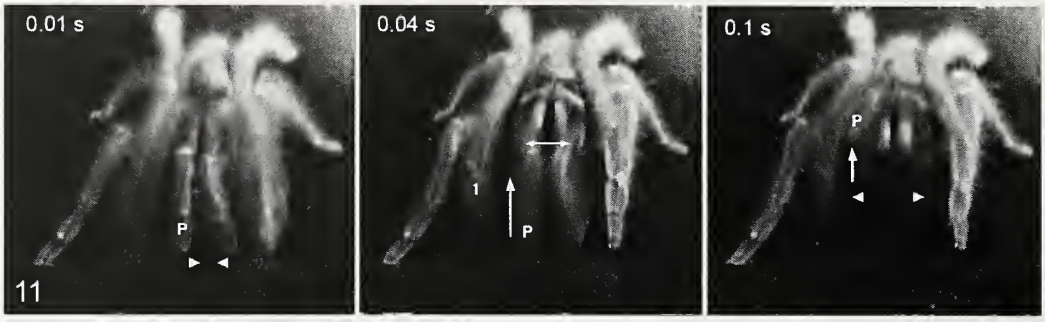

Figure 11.—Three consecutive still pictures from a video film of the defensive reaction in *Ephebopus cyanognathus*. Left: Immediately following a mechanical stimulus, both palps (P) are held close together (arrowheads) and are touching the substrate. Middle: Only 40 milliseconds later, the first legs (1) and the palps (P) are rapidly moved upward (vertical arrow); at the same time, the chelicerae are spread apart (horizontal arrows), thus exerting more friction upon the pedipalpal brushes on the femora. Right: The palps are now in an Up-Position and kept much farther apart (arrow heads). It is at this point that a small puff of urticating hairs is released.

In order to determine how many urticating hairs there are within a palpal brush, it is best to examine areas where the urticating hairs have been brushed off and only the empty sockets remain. The sockets are rather evenly spaeed at a distance of about 20 µm, a bit closer in the vertical direction than in the horizontal (Fig. 3). On scanning electron micrographs we counted 55 sockets on an area of 0.01 mm^2, which corresponds to a density of 5500 urticating hairs/mm^2. Since the entire palpal brush measures around 1.5 mm^2, the total number of urticating hairs on one palp is about 8250.

How does *Ephebopus* actually release the urtieating hairs from its palpal brushes? Is it an interaction between the two palps or between the palps and chelicerae? Since the defensive reaction (palpal flicking) happens so fast, we used a video camera and analyzed the movements of the involved body parts in consecutive frames.

At the onset of the defensive reaction, the spider holds both palps close together, touching the substrate (Fig. 11, left). About 30–40 ms later the first legs and the palps are rapidly thrust upward and at the same time the chelicerae are spread sideways (Fig. 11, middle). This apparently creates friction between the inside of the femora (pedipalpal brush) and the outside of the chelicerae. After 100 ms the palps are seen in the Up-position and are held much farther apart than at the beginning (Fig. 11, right). It is at this moment that a little puff of urticating hair is released into the air. The entire defensive reaction consists of only one upward stroke of the palps that lasts approximately 0.1 s. Most of the time, the spiders are reluctant to repeat this behavior and instead tend to flee.

DISCUSSION

The previous descriptions of the urticating hairs in *Ephebopus* sp. (Marshall & Uetz 1990; West et al. 2008) were very brief and did not give any dimensions. We found that most hairs are 500–600 µm long but only 5–6 µm in diameter; thus the shape corresponds more to a long knitting needle, which is in contrast to the description "*short and stout*" given by Marshall & Uetz (1990). Only the rather long and thin urticating hairs, with a length to diameter ratio of 100:1 or 200:1, are considered to float through the air (Bertani & Marquez 1995/96).

Barbs along the urticating hair shaft pointing distally have been discussed with regard to other tarantulas, and it was argued that the acute distal end could not be the penetrating tip (Bertani et al. 2003; Pérez-Miles 1998). This makes sense, since the barbs would quickly get stuck in the skin if pushed in the wrong direction. The basal end of the hair is less pointed but very thin (2 µm) and squared off obliquely like a hypodermic needle. One could well imagine that the basal end functions as the penetrating tip, yet experimental proof is lacking so far.

Urticating hairs in tarantulas were classified into four different types by Cooke et al. (1972), based on morphological differences. Marshall and Uetz (1990) added a "fifth type" for *Ephebopus*, but did not describe the typical features. Our definition of type V urticating hairs would read as follows: Straight hairs of 0.5 mm length and 5 µm diameter, slightly bent at the base (socket region), fine barbs (0.5 µm) along the entire hair shaft pointing toward the distal end. Overall, type V hairs are similar to type II urticating hairs, except for the distinct basal stalk of the latter.

Based on the density of empty sockets we calculated about 5500 urticating hairs for 1 mm^2, or around 8250 hairs for one palpal brush. This seems a modest number when compared to over 10,000 hairs/mm^2, as was claimed for abdominal urticating hairs (Cooke et al. 1972). Unfortunately, the authors did not provide any measurements for the diameter of those hairs (type I), but deducing from their mierographs, it should be around 7 µm. In order to achieve that high density the hair sockets would have to be spaeed at 10 µm or less. Since this leaves almost no space between the hair shafts, it would mean a veritable "solid forest" of urtieating hairs. In *Ephebopus*, soekets are spaced at 20 µm, which itself makes for a very dense packing (Fig. 3).

It was surprising to find only small denuded areas (with empty sockets) within the palpal brushes of the exuvia. It could be that these spiders had hardly ever defended themselves, or that they shed only a very limited amount of urticating hairs during one palpal flick. It seems necessary, of course, that *Ephebopus* be thrifty with its urticating hairs, since they can grow back only between molts. In those animals filmed repeatedly for their defensive reaction, the palpal brushes showed large denuded patches. We estimated that those spiders had reaeted with a palpal flick approximately 5–10 times. In one of these animals we found almost 5000 empty sockets on the proximal side of each palpal brush. This would correspond to more than 500 urticating hairs released with a single palpal flick.

Another interesting aspect is that the palpal flick has to be a controlled action; otherwise all urticating hairs might get lost

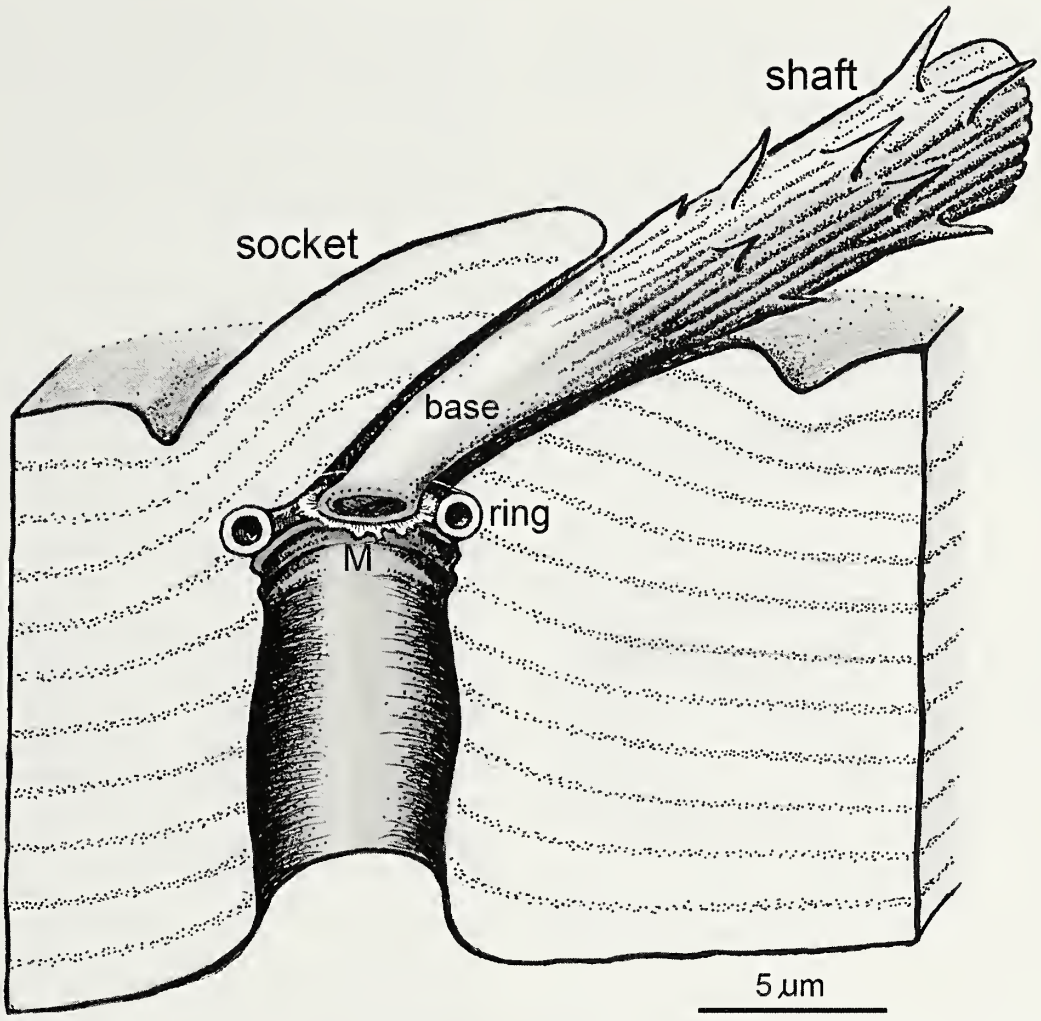

Figure 12.—Cut-away view of the attachment of an urticating hair in *Ephebopus cyanognathus*. The hair is only held by a thin membrane (M) between the hair base and a ring-like structure beneath the socket. This is the site of detachment when the urticating hair is released.

with one vigorous stroke. A mechanical feedback indicating the number of urticating hairs could be transmitted by sensory hairs that are interspersed among the palpal brush. These hairs can hardly be seen in an intact hair field, but stand out in denuded areas. In contrast to the urticating hairs, they remain in their sockets after a palpal flick.

The main questions of this study were: how are those urticating hairs attached, and how can they be detached? The hair shaft tapers down to 2–3 µm near the base and then disappears in a tightly fitting socket (Figs. 2, 3). Initially we assumed that any forcible movement of the hair against the rim of the socket would break the hair shaft right there and leave a little stump inside the socket (Foelix et al. 2009). However, this is not the case. All released hairs remain practically in one piece and there are no stumps in the sockets. So, how is the hair base actually connected inside the socket? We were expecting to get an answer from longitudinal sections of palpal brushes embedded in hard Epon. Unfortunately this failed because during sectioning with the microtome the embedding medium separates from the leg cuticle and practically all urticating hairs are torn out (Fig. 4). We were more successful by simply cutting strips out of the pedipalpal brushes with a razor blade and then inspecting those longitudinal sections under the scanning electron microscope. Again, most sockets were empty due to the manipulation with the razor blade, but if the sockets were only grazed by the blade, the hairs remained in place (Figs. 9, 10). In such cases it was clearly seen that the hair base is connected by a very thin cuticular membrane to a ring structure lying horizontally beneath the socket. This delicate attachment is apparently the only connection of the urticating hair to the socket (Fig. 12). In contrast, sensory hairs were found to be held by strong joint membranes in their sockets; additionally, they also have a fine connecting membrane at the very base of the hair (Fig. 6). It thus seems that it is the lack of a joint membrane that makes it easy for urticating hairs to become detached.

How does *Ephebopus* actually release its urticating hairs? The only description available states that spiders "*were observed to bring the pedipalps down across the basal segments of the chelicerae in a brief scrubbing motion*" (Marshall & Uetz 1990). Our analyses show that it is indeed a single motion; however, it seems to be the upstroke of the palps against the spread chelicerae that causes the release. The entire reaction lasts only 0.1 s.

It is also interesting that there is no obvious counterpart present on the chelicerae, such as marked ridges or a comb that

would scrape the urticating hairs out of the pedipalpal brush. On the contrary, the lateral surface of the cheliceral basal segment is surprisingly smooth. Perhaps this is actually an advantage, so that the barbed little "spears" will not get caught on the spider's body but can fly off into the air more easily.

Finally, we must raise the question of why *Ephebopus* is the only genus so far known to have urticating hairs on the palps rather than on the abdomen. For a tube-dwelling tarantula it certainly makes sense to defend itself frontally toward an aggressor, because any attack would usually occur from in front. However, there are many other Neotropical tube-dwelling tarantulas that first have to turn around and then defend themselves by brushing off their abdominal urticating hairs with their hind legs. And apparently they do so quite successfully. Unfortunately, we do not know how and when *Ephebopus* makes use of its urticating hairs under natural conditions.

ACKNOWLEDGMENTS

Sam Marshall and George Uetz shared their experience with *Ephebopus* urticating hairs with us. Gianni Sposato kindly lent us his video camera. Martin Huber helped us with the literature search and Jerome Rovner critically read the manuscript. Benno Wullschleger and Jan de Vries assisted us in technical matters. We are grateful to all these people.

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Possible niche differentiation of two desert wandering spiders of the genus *Syspira* (Araneae: Miturgidae)

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Abstract. When species with similar morphological and ecological characteristics occupy the same habitat, selection should minimize resource competition and promote coexistence by means of spatial partitioning. Competing species might exploit resources at different times or specialize in distinct resources. From July 2005 through May 2006 we examined the niche axes of two endemic sympatric desert species, *Syspira tigrina* Simon 1885 and *Syspira longipes* Simon 1885 in the State of Baja California Sur, looking for evidence that coexistence is fostered by differences in choice of microhabitat, temporal activity, occupation of space, or size. The results show high monthly microhabitat overlap (> 0.9). However, we found subtle differences in temporal activity and marked differences in juvenile and male body size, as well as some evidence of mutual spatial segregation. We conclude that body size and spatial segregation appear to be the dominant niche axes that facilitate coexistence of these species.

Keywords: Ecological segregation, morphological segregation, niche overlap

A central goal of ecology is to understand the forces that maintain species diversity within communities (Hutchinson 1959; Pacala & Tilman 1993; Chesson 2000). Hardin (1960) suggested that sympatric similar species competing for the same resources cannot stably coexist because one species will always be more efficient than the others and will quickly drive them to extinction. Consequently, coexistence requires some form of resource partitioning between co-occurring species to reduce or prevent interspecific competition (Amarasekare 2003). Hutchinson (1959), Chesson (2000), and Davies et al. (2007) state that partitioning can occur in three ways. First, species might differ in where they experience and respond to a limiting factor (spatial habitat partitioning). Second, different species may be limited by the same resources, but differ in the time when they exploit the resource (temporal partitioning). Third, co-occurring species may specialize in different resources (resource partitioning). These kinds of partitioning would be the result of selection for ecological character divergence among sympatric populations (Brown & Wilson 1956; Dayan & Simberloff 2005; Davies et al. 2007).

Different types of segregation have been reported among coexisting spiders. Among diurnal sympatric web-builders, scientists have observed a clear microhabitat segregation pattern in tetragnathids and linyphiids (Aiken & Coyle 2000; Wright & Coyle 2000). Henaut et al. (2001) have reported prey partitioning among araneids. Among nocturnal sympatric araneids and tetragnathids, researchers have noted temporal and spatial segregation (Ward & Lubin 1992).

Among diurnal wandering spiders, Uetz (1977) and Turner & Polis (1979) found seasonal specialization to be the predominant niche dimension facilitating coexistence of some gnaphosoids, while spatial (Suwa 1986) and microhabitat segregation (Moring & Stewart 1994; Carrel 2003) were the key to coexistence in some lycosids. Cutler & Jennings (1992) found that habitat partitioning is common among congeneric jumping spiders. Among syntopic, congeneric, nocturnal wandering spiders, only ctenids have been studied, and the results suggest that among *Ctenus* species there is no clear niche partitioning (Gasnier & Höfer 2001), but that *Cupiennus* species could be separated by differences in phenology on a seasonal basis (Schuster et al. 1994).

In the desert of Baja California Sur, there are two sympatric *Syspira* species: *Syspira tigrina* Simon 1885 and *S. longipes* Simon 1885 (Araneae: Miturgidae). These endemics (Olmstead 1975), are medium sized (8–12 mm) ground-dwelling spiders that are active during the night. Pitfall trap collections indicate that they represent up to 50% of all wandering spiders where they are found, so they are an important component of the desert ground spider assemblage (Nieto-Castañeda 2004). This genus has not yet been used as a model in ecological studies.

By documenting small-scale patterns of sympatric populations of *S. tigrina* and *S. longipes*, we wished to look for patterns of microhabitat occupation, microhabitat overlap, spatial segregation, temporal activity, and size segregation over a span of one year. Specifically, we asked the following questions: 1) What is the structural microhabitat occupied by the two species? 2) Is there evidence of overlap in microhabitat? 3) Are there indications of mutual spatial exclusion? 4) Is there evidence of temporal separation in activity? 5) Are there indications of size segregation? The answers to these questions will help us understand how co-occurrence of these two congeners takes place.

METHODS

Study area.—We selected four localities in the southern extension of the Sonoran Desert (León de la Luz et al. 2000) to represent a diversity of ground habitats, including three oases with varying availability of water and one locality without a water source. The oases are Presa de la Buena Mujer (24°05′N, 110°11′W, 180 m a.s.l.), a reservoir; Laguna San Pedro (23°56′N, 110°09′W, 6 m a.s.l.), a lagoon on the Pacific coast; El Novillo (23°55′N, 110°13′W, 220 m a.s.l.), a small pond in the hills, and El Comitán (24°07′N, 110°25′W, 20 m a.s.l.), a dry area (Fig. 1). The region is a subtropical desert with hot

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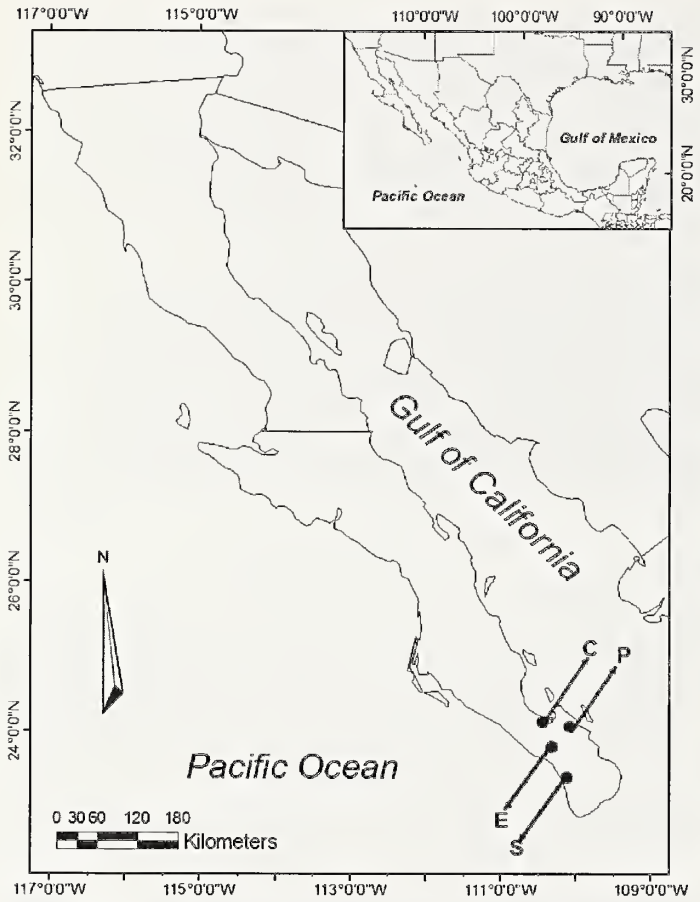

Figure 1.—Map of study sites where the two *Syspira* spider species were collected. S: Laguna San Pedro, P: Presa de la Buena Mujer, E: El Novillo, C: El Comitán.

summers and a sporadic rainy season between July and October, and warm winters with little or no rain between November and February. The vegetation is subtropical desert shrub (León de la Luz et al. 1996).

Field work.—At each site, we plotted two belt transects (100 m × 1 m), separated by 50 m. Transects were divided into 20 quadrants (5 m × 1 m). We only sampled odd-numbered quadrants to avoid disturbance to the contiguous ones. We sampled the same quadrants every time because sizes of monthly catches of *Syspira* did not decline over a year (Nieto-Castañeda 2004) in a previous study of Baja California's wandering ground spiders using pitfall traps. Exhaustive hand collections with headlamps were made by a three-member team, which spent one night at each locality every 3 mo from July 2005 through May 2006. This sampling pattern included two rainy seasons (July and January) and two dry seasons (October and May), totaling 16 collecting days. Sampling started at dusk when *Syspira* spiders become active and continued for 4–5 h, until spiders were no longer present. The spiders were preserved in 70% ethanol.

Characterization of microhabitats.—When a spider was found, we drew a 0.19 m^2 circle (based on a 25 cm radius) around it and measured ten variables to characterize the microhabitat. The percentage of the following five ground surfaces was identified: (1) bare soil, (2) fallen logs and branches, (3) leaf litter and twigs, (4) gravel and pebbles (2–64 mm dia.), and (5) cobbles (64–256 mm dia.). Next, we

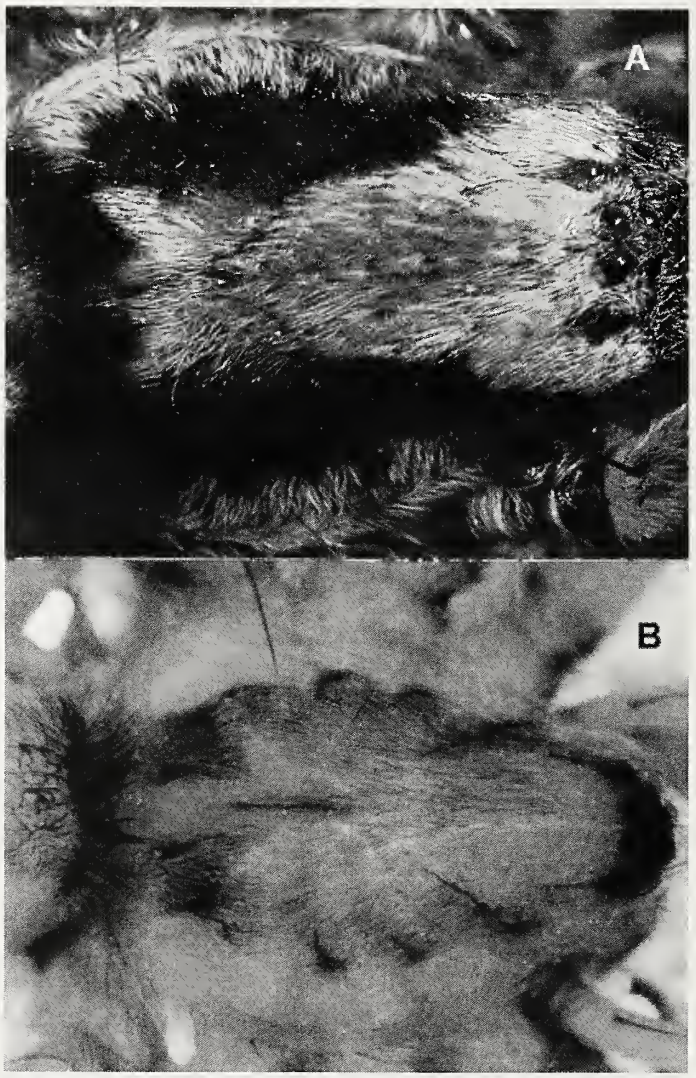

Figure 2.—Dorsal view of carapace pattern of adults of *S. tigrina* (A) and *S. longipes* (B).

immediately estimated the percentage of above-ground vegetation (6). We then recorded (7) plant life forms (trees, shrub, herbs), (8) soil texture [measured as categorical variable 1: 100% of sand, 2: <100, and >70% of sand, 3: <70% of sand) by the wet feel method (Thien 1979)], (9) temperature, and (10) relative humidity at the bare soil surface with a thermo-hygrometer (HI-8564, Hanna).

Species identification and measurements.—All spiders were identified to species level using Olmstead (1975) and then sexed if they were mature. We included the immature spiders because they are necessary for any objective community analysis (Sackett et al. 2008). These spiders, even young specimens, are easily identified by the markings on the carapace. *S. tigrina* has three dark stripe markings: two are longitudinal and almost parallel, beginning near coxa I and ending before the posterior edge of the carapace; these stripes are separated by at least the distance between the anterior lateral eyes. The third stripe is perpendicular to the other two and is closer to the posterior edge of the carapace (Fig. 2A). *S. longipes* has reticulated markings (Fig. 2B).

The total tibia I length and the carapace width of every spider was measured as an indicator of body size (Hagstrum

Table 1.—Number of juveniles (J), adult males (M), and adult females (F) of two *Syspira* species sampled each month in study.

Species	July			October			January			May			Total
	J	M	F	J	M	F	J	M	F	J	M	F	
S. tigrina	59	11	18	76	4	1	105	0	4	56	2	3	339
S. longipes	21	8	2	27	0	3	21	0	1	7	1	2	93

1971; Toft 1976). We did not measure total body length because that can change quickly with alterations in foraging success. Measurements were performed with a stereoscopic microscope, using a micrometer. Voucher specimens were deposited in the Arachnological and Entomological Collection of the Centro de Investigaciones Biológicas del Noroeste (CAECIB).

Data analysis.—*Characterization of microhabitat:* To test whether species occupied different microhabitats with respect to the month sampled, we used principal components analysis (PCA) with varimax rotation of the correlation matrix. This reduced the ten continuous microhabitat variables to a smaller number of variables that explained most of the variation in the raw data. Prior to conducting the PCA, we $\log_{10}$-transformed temperature and relative humidity, after adding 1 to improve normality and reduce heteroscedasticity. Then percentages were converted to proportions and transformed by arcsine square root (Goodman 2007). PCA was separately conducted for each season, which generated four sets of PC axes. PC axes with eigenvalues greater than 1.0 and eigenvectors with scores greater than 0.7 were considered informative. The first two principal components were plotted against each other to find structure in the data that could distinguish *S. tigrina* specimens from *S. longipes* specimens. All analyses were performed with STATISTICA v. 6.0 software (StatSoft, Inc).

Overlap of microhabitat: Using the most informative variables in PCAs, we calculated Pianka's Index of microhabitat overlap by sampled month with the ECOSIM v. 7.0 software (Gotelli & Entsminger 2008). We then determined the statistical significance of the observed microhabitat overlap by comparing it with the RA3 algorithm, where the niche breadth was retained and the zero states were reshuffled. In the monthly presence-absence matrices, each row represented the two *Syspira* species and each column represented a different microhabitat category, in which the observed data on resource utilization were randomized between the two species in 5000 simulations with proportional representations of the two *Syspira* species and resources (Gotelli & Entsminger 2008).

Spatial segregation: With an abundance matrix by sampled month in place, where rows represented the two *Syspira* species, columns represented different quadrants (80 by month, 320 in total), the co-occurrence module of ECOSIM v. 7.0 was used to test for non-random patterns of species co-occurrence. We calculated co-occurrence scores (C-score) as the numbers of checkerboard units, based on 5000 interactions with proportional representations of species and quadrant sites. Species representations (rows) and quadrant location representations (columns) were kept 'proportional' because these conditions best reflected differences between species in terms of trapping and spatial heterogeneity in trapping probability. We calculated the expected C-scores (null models) and subsequently tested for whether the occurrence of *S. tigrina* and *S. longipes* deviated from random occurrence (Gotelli & Entsminger 2008).

Temporal activity pattern: We used a Fisher exact test by species to test for independence of spider abundance between transects. We then performed a chi-squared goodness of fit test to determine monthly differences in species abundance. All analyses were performed with the Stata v. 9.1 software (StataCorp, College Station, TX).

Size segregation: We also conducted a multivariate two-group Hotelling's T-squared test with Stata v. 9.1 software, which tested for significant monthly size differences in the carapace width and total tibia length between *Syspira* species by developmental stage. Statistical significance was set at $P < 0.05$.

RESULTS

Spiders collected.—During this study, we collected 432 *Syspira* spiders. Immature spiders represented almost 87% of all *Syspira* spiders and were more abundant in January, while adults were more abundant in July. *S. tigrina* was the most abundant species in every month (Table 1).

Characterization of microhabitat.—PCAs with microhabitat variables organized by month were used to characterize the microhabitats occupied by each species. The first two components of the four PCAs accounted for ~ 50% of the variation (Table 2). *S. longipes* were always within the microhabitat conditions occupied by *S. tigrina*. Positive scores for PC1 correlated with moist areas during July, October, and January and dry areas during May. Negative scores for PC1 were associated strongly with cool areas in July, October and January and warm areas during May. According to PC1, *S. longipes* was restricted to the areas that were cooler and had higher relative humidity than areas occupied by *S. tigrina* during July and January and to the areas that were warmer and had lower relative humidity occupied by *S. tigrina* during October and May. During May, positive scores were linked with low sand soil texture. There were slight differences in the structural microhabitat variables correlated with PC2. During July, positive scores showed a relationship with higher bare soil surface, negative scores were correlated with higher leaf litter and twigs surface. During October and January, positive scores for PC2 were interconnected with high leaf litter and twig surface, and negative scores correlated with high bare soil surfaces. During May, PC2 was positively correlated with vegetation coverage above ground and life form, but PC2 did not distinguish *S. longipes* from *S. tigrina* (Fig. 3).

Overlap of microhabitat.—Monthly microhabitat overlap for both species was high, indicating an almost complete overlap. This result was significantly higher than expected by chance ($P \leq 0.05$) (Table 3).

Spatial segregation.—The two species shared only a small number of the 320 quadrants in all months, and most

Table 2.—Correlations of ten structural microhabitat variables with the first two axes obtained from PCA (PC1, PC2) for each month. Bold numbers indicate the most important variables. Eigenvalues and total variance explained are provided too.

	July		October		January		May	
Variable	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Bare soil	0.38	**0.86**	−0.08	**−0.91**	0.26	**−0.91**	0.00	−0.06
Fallen logs and branches	−0.09	0.00	0.39	−0.28	0.05	0.19	0.02	−0.52
Leaf litter and twigs	−0.02	**−0.84**	0.01	**0.90**	0.14	**0.95**	−0.19	0.16
Gravel and pebbles	−0.48	−0.22	−0.17	−0.06	−0.73	−0.03	0.24	0.34
Cobbles	−0.31	0.01	0.50	0.12	−0.25	−0.07	0.12	−0.49
Above ground vegetation	0.56	−0.33	0.03	0.07	0.10	0.16	−0.29	**0.69**
Life form	0.21	−0.57	0.09	0.19	0.13	0.22	0.05	**0.77**
Soil texture	−0.65	−0.09	−0.39	0.21	−0.24	0.23	**0.74**	−0.19
Temperature	**−0.76**	0.15	**−0.84**	0.00	**−0.83**	0.03	**0.95**	0.01
Relative humidity	**0.84**	−0.11	**0.88**	0.10	**0.88**	−0.09	**−0.91**	0.04
Eigenvalue	2.55	1.98	2.38	1.89	2.70	2.17	2.92	1.70
% Total variance	25.50	19.77	23.84	18.93	27.00	21.69	29.16	17.03

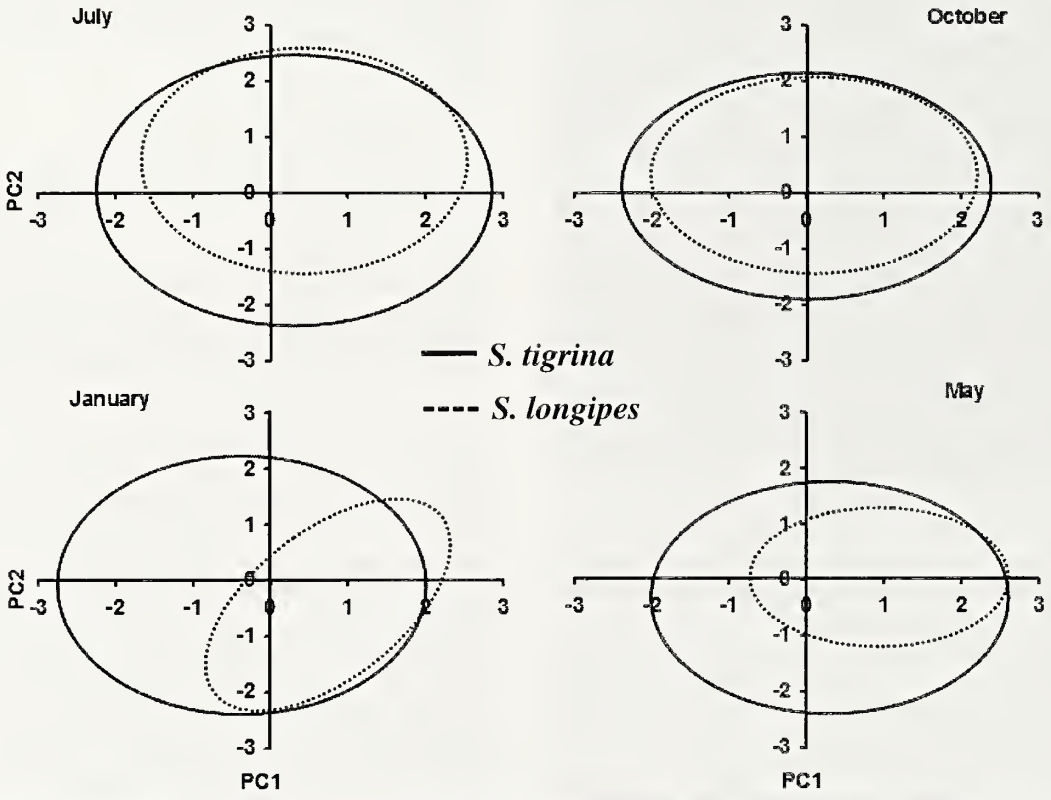

Figure 3.—Structural microhabitat occupied by two *Syspira* species each month in two-dimensional ecological space based on principal components scores (PC1 and PC2).

Table 3.—Observed and expected Pianka's overlap indices for each month. Indices are given as the mean ± SD. Expected values are based on 5000 interactions with proportional representation of species and resources.

	Overlap Index	
Month	Observed	Expected
July	0.95	0.79 ± 0.08
October	0.97	0.75 ± 0.09
January	0.94	0.73 ± 0.09
May	0.92	0.87 ± 0.03

quadrants were exclusively occupied by one species. In all months, C-scores were significantly higher than expected by chance, which indicated interspecific spatial segregation ($P \leq 0.05$) (Table 4).

Temporal activity pattern.—We found that *S. tigrina* had significantly different patterns between transects ($\chi^2_3 = 20.79$, $P < 0.05$), but *S. longipes* did not; neither species had the same monthly abundance pattern ($\chi^2_3 = 4.21$, $P > 0.05$) (Fig. 4).

Size segregation.—Juveniles of neither species had the same average tibia and carapace size among months (July: $F_{2,77} = 7.38$, October: $F_{2,100} = 40.77$, January: $F_{2,123} = 60.02$, May:

Table 4.—Frequency of quadrants (for each month = 80; total = 320) occupied by one, both, or neither *Syspira* species. Observed and expected C-scores are given. Indices are given as the mean ± SD. Expected values are based on 5000 interactions with proportional representation of species and trap quadrants.

	Species in quadrants			C-score	
Month	None	Single	Both	Observed	Expected
July	31	41	8	310	111 ± 41
October	32	34	14	208	96 ± 35
January	28	44	8	259	108 ± 46
May	40	36	4	155	59 ± 32

$F_{2,60} = 12.96$, $P < 0.05$). Females of neither species exhibited significantly different tibia and carapace sizes in most months (October: $F_{2,1} = 0.02$, January: $F_{2,2} = 0.23$, May: $F_{2,2} = 0.96$, $P > 0.05$) except in July ($F_{2,17} = 6.35$, $P < 0.05$). Males of both species had significantly different tibia and carapace sizes in July ($F_{2,16} = 20.10$, $P < 0.05$), but in the other three months the sample size was too small to test. *S. longipes* spiders had a longer tibia I and a wider carapace than *S. tigrina* in all stages and months (Fig. 5).

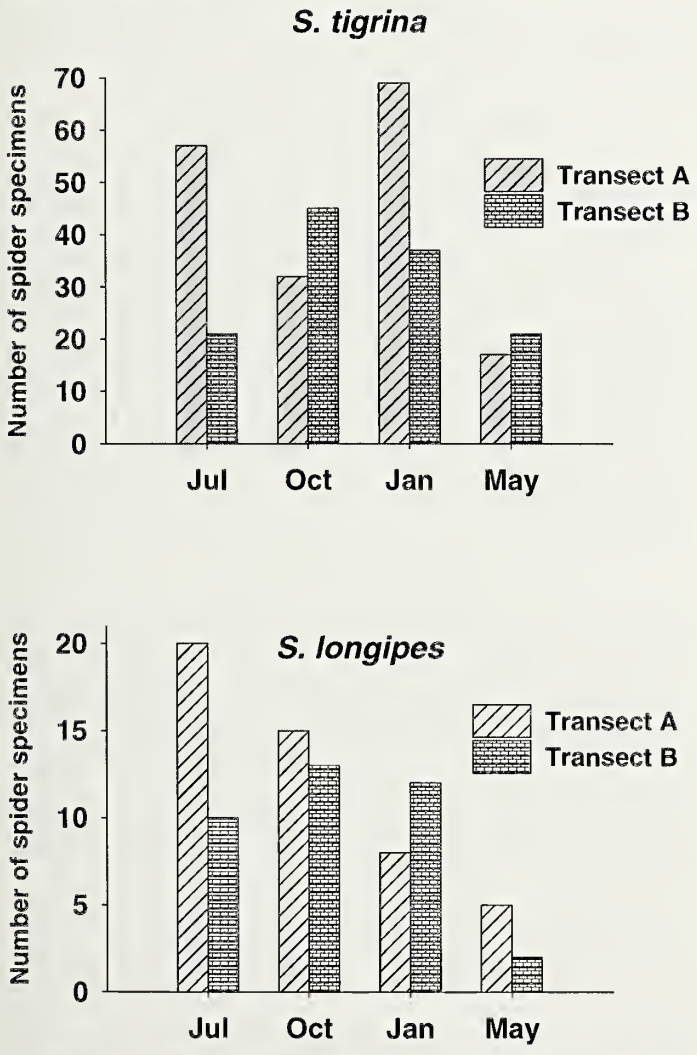

Figure 4.—Monthly numbers of active *Syspira* species in two transects (A, B) sampled at each site.

DISCUSSION

Large similarities in microhabitat occupancy indicate that *Syspira* species should compete intensely (Holt et al. 1994), with limited species overlap (Goodman 2007). Additionally, members of the species should segregate in other dimensions, as has been observed for other wandering spiders (Schuster et al. 1994; Gasnier & Höfer 2001). In co-occurring, congeneric, web-building spiders of the families Araneidae, Tetragnathidae, and Linyphiidae in Nearctic regions, microhabitat segregation appears to be a main factor allowing coexistence (Aiken & Coyle 2000; Wright & Coyle 2000). This type of segregation between congeneric species has been documented in burrowing wolf spiders of the genus *Geolycosa* in Florida (Marshall et al. 2000; Carrel 2003) and ground-wandering *Pardosa* species in Japan (Suwa 1986). There is, however, no evidence of habitat segregation among nocturnal, congeneric, terrestrial cursorial spiders.

The constrained occupancy of the microhabitat of *S. longipes* relative to *S. tigrina*, with respect to temperature and relative humidity, suggests possible differences in their metabolism, since these environmental factors affect spider performance (Huey & Kingsolver 1989). Spatial arrangements of *Syspira* species appear unrelated to leaf litter and twigs or bare surfaces, even in desert communities that lack much structural complexity, although these features are considered to be one of the most critical microhabitat variables affecting community structure (Melville & Schulte 2001; Goodman 2007).

We were not surprised to find significant spatial segregation between these *Syspira* species during all months since there are several studies suggesting that similar sympatric spider species differ principally in spatial distribution. For example, four wandering *Ctenus* species in Central Amazonia segregate spatially (Gasnier & Höfer 2001) and four sympatric orb weavers (Araneidae and Tetragnathidae) that inhabit coffee plantations in Mexico reduce competition by building webs of varying structures in different locations (Henaut et al. 2001). Congeneric species of *Pardosa* have the same daily and seasonal pattern, but segregate by vertical or horizontal stratification (Greenstone 1980; Suwa 1986).

As expected, we found slight differences in temporal segregation; however, it has not been well documented in congeneric wandering spiders, although Turner & Polis (1979) and Uetz (1977) stated that temporal segregation was an important factor in reducing niche overlap. Yet this type of segregation has been reported in other co-ocurring species, namely *Pardosa milvina* (Hentz) with *Hogna helluo* (Walckenaer) on soybean farms in Ohio (Marshall et al. 2002). Ward & Lubin (1992) found that six nocturnal orb-weavers (Tetragnathidae and Araneidae) occupied the same habitat, but had different daily and seasonal activity patterns.

Competition for food has long been considered a keystone of community ecology. Therefore, differences in the average monthly size of juveniles, and sometimes adults, of both species may enable trophic divergence because body size is a reliable determinant of prey size, non-web-building spiders typically consuming prey of similar size to themselves (Gertsch & Riechert 1976; Nentwig & Wissel 1986). The significant overlap in average body size between females of

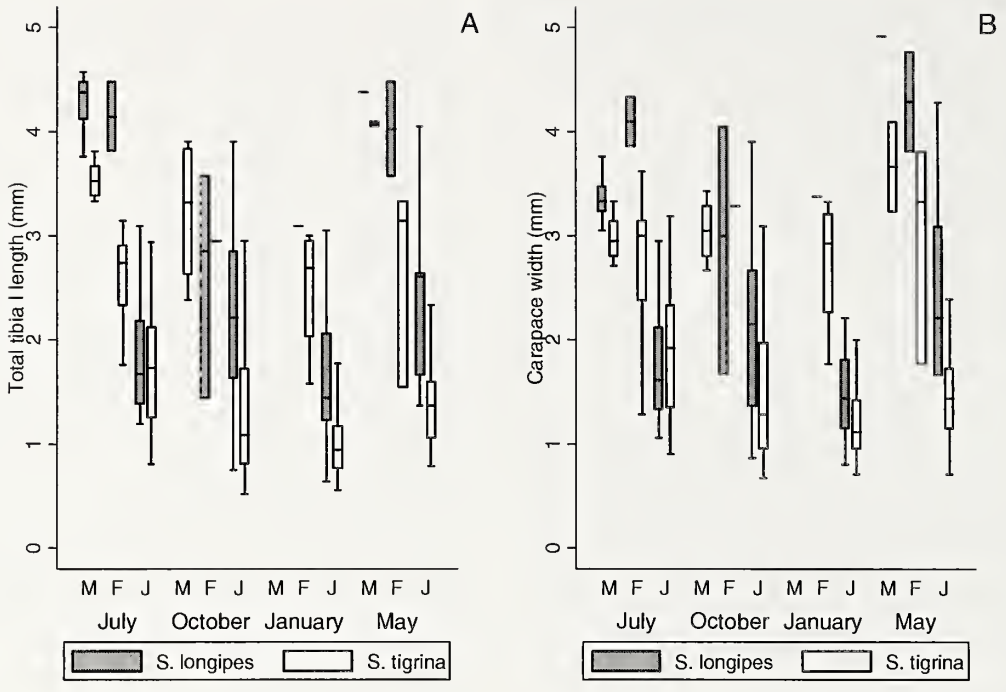

Figure 5.—Box plots of tibia I length (A) and carapace width (B) of both *Syspira* species. M: males, F: females, J: juveniles.

both species from October to May suggests that competition for prey or other resources may be high. There are many cases among animals that highlight the important role of phenotypic divergence between sympatric species as a way to avoid competition for food: bill size and shape in passerine birds (Newton 1967); body size among amphibians, reptiles, insects, and rodents; canine teeth diameter in carnivores (Pimm & Gittleman 1990); and neck height or incisor arcade structure in herbivorous mammals (Gordon & Illius 1988; Du Toit 1990).

Differences in size may also be attributable to character displacement (Guilleman et al. 2002; Dayan & Simberloff 2005). Such displacement occurs when selection, during extended periods of sympatry among animals that partition resources, results in an accumulation of morphological differences that reduces or resolves competition. However, such differences in morphology may have arisen before the species came into sympatry, and these differences may have been responsible for facilitating coexistence at its initial stages. In either case, morphological distinctiveness is likely to be a major contributor to stable coexistence of potential competitors (York & Papes 2007). Few studies actually address phenotypic divergence between sister sympatric species. Many researchers assume that closely related species are more likely to compete than distantly related ones (Dayan & Simberloff 2005). There are no studies in which phenotypic differences among sympatric spider congeners are correlated as a consequence of character displacement.

These two *Syspira* are closely related sympatric species that have probably evolved similar life histories, and, since they use the same microhabitats, have similar resource use. The spatial, temporal (to a lesser degree), and size differences between the two species may be the key factors permitting their coexistence.

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Construction and function of the web of *Tidarren sisyphoides* (Araneae: Theridiidae)

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Abstract. In this paper, we describe the construction and function of the double sheet and tangle web of *Tidarren sisyphoides* (Walckenaer 1842). Web construction includes several stages: construction of the scaffolding that serves to support the rest of the web; filling in the dome-shaped and horizontal sheets; and construction of the upper tangle. During construction of the scaffolding, the spider descends by a pre-existing thread to the substrate, moves a few centimeters and attaches the dragline, then she ascends by the new thread, doubling the line or attaching it to another thread. The spider fills in the sheet while walking in an irregular pattern under the sheet, and attaching her dragline using either one or both legs IV simultaneously to hold pre-existing sheet lines against her spinnerets. During scaffolding construction and filling in the dome-shaped sheet, the spider returns frequently to the retreat, apparently using the same threads near the retreat each time. Threads of both the dome-shaped sheet and the horizontal sheet have small drops of viscid material. The dome-shaped sheet and upper tangle comprise the functional trap of the web, while the horizontal sheet apparently plays only a little role in prey capture.

Keywords: Web-building behavior, aerial sheet web, web function, viscid threads

Web designs in Theridiidae are strikingly variable (Szlep 1965, 1966; Lamoral 1968; Eberhard 1972, 1981, 1991; Agnarsson 2004, 2005, 2006; Eberhard et al. 2008a), and similar designs have evolved independently in different genera, and in different species within a genus (Darchen & Ledoux 1978; Eberhard 1991; Japyassú & Jotta 2005; Barrantes & Weng 2006a, 2007; Jörger & Eberhard 2006; Eberhard et al. 2008a). The broad disparity in theridiid webs is possibly the result of their great flexibility in microhabitat use, their ability to adjust web design to different physical spaces, prey types, and prey availability (Turnbull 1964; Eberhard 1990a; Agnarsson & Coddington 2007; Jörger & Eberhard 2006; Eberhard et al. 2008b), and their response to parasitism and predation pressures (Blackledge et al. 2003; Agnarsson 2004; Barrantes et al. 2008).

Webs of theridiid spiders are sometimes described as an irregular, three-dimensional structure (Foelix 1996). However, their webs range from those that are extremely simplified as in *Phoroncidia studo* Levi (Eberhard 1981), with a web consisting of a single sticky line, to extremely complex, three-dimensional webs with aerial sheets, as in *Achaearanea disparata* Denis 1965 (Darchen & Ledoux 1978) and *Tidarren sisyphoides* (Eberhard et al. 2008a). Despite the diverse array of web designs and the convergence in some of these designs, the detailed descriptions of the web-building behavior have begun to reveal some patterns in the typical behavior used to manipulate lines and in the sequence of lines laid (Benjamin & Zschokke 2003; Jörger & Eberhard 2006; Eberhard et al. 2008b). Knowledge of how three-dimensional webs of theridiids are built is generally fragmentary (Szlep 1965, 1966; Lamoral 1968; Benjamin & Zschokke 2003), and limited to only a few genera.

All webs described for species within the derived genus *Tidarren* are tangles with aerial sheets (Agnarsson 2004; Benjamin & Zschokke 2003; Eberhard et al. 2008a). Those of *T. sisyphoides* (Walckenaer 1842) (Benjamin & Zschokke 2003) and *Tidarren* spp. (see Agnarsson 2004) have been described as lacking viscid threads. The sheet of *T. sisyphoides* is dome-shaped, with a relatively dense tangle above it (Eberhard et al. 2008a). The spider hides in a retreat, often a curled leaf, suspended in the tangle at the peak of the dome, opening onto the underside of the dome (Eberhard et al. 2008a). Web construction behavior has never been described in *Tidarren*. The only report of construction of an aerial sheet web is for *Achaearanea tesselata* (Keyserling 1884) (Jörger & Eberhard 2006). This study describes the web construction behavior of *T. sisyphoides*, and the function of the areas of its web.

METHODS

We observed web construction behavior of 15 adult female *T. sisyphoides* indoors in wire cubes 30 cm on a side, hanging 2 m above the floor from a thin fishing line. The cubes had a wire along each of the diagonals at the top, and one along one of the diagonals at the bottom. We collected the spiders with their retreats on the campus of the Universidad de Costa Rica, San Jose province (9°54′N, 84°03′W), Costa Rica. We hung each retreat individually from the intersection of the two diagonal wires at the top, using silk threads (4–5 cm long) of the same web. Two spiders did not use the retreats and constructed webs without them.

We photographed twelve webs each day for several days (digital camera Olympus SP-510UZ), until each web was completed. Webs were sprayed with water just before photographing them to create a better contrast of silk threads against the cubes' backgrounds. We video recorded the complete construction of three additional webs using a Sony digital camera DCR-HC 96. Recording distance from the spider was intentionally changed during web construction to have either the entire cube in view or close-ups of different construction behaviors. We searched for sticky droplets on threads in five webs from the field. Thread samples from sheets were collected on slides framed with strips of double-sided adhesive tape, and density of viscid globules was measured following Barrantes & Weng (2006b). We photographed viscid globules present in these threads under a compound microscope (digital camera Nikon Coolpix 4500) with a relative humidity of 60%. Viscid globules were then placed in a saturated humidity chamber for 40 min and observed under the dissecting microscope for changes in size.

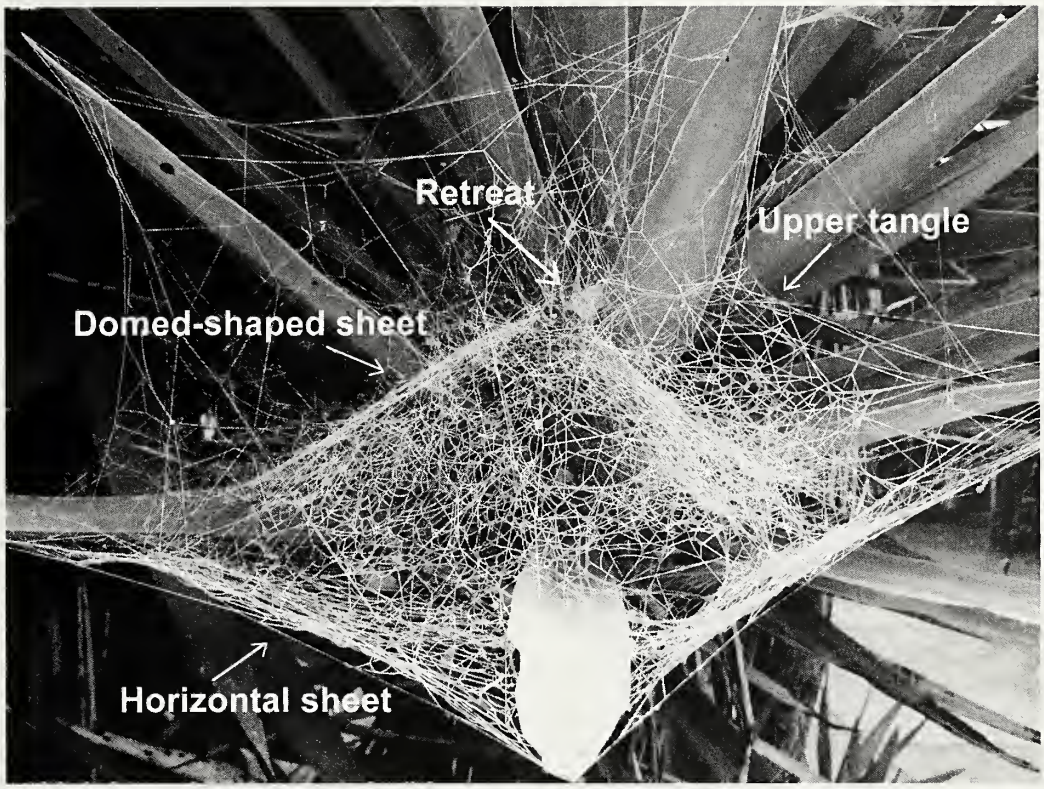


Figure 1.—Web of *Tidarren sisyphoides* (powdered with talcum) showing the upper tangle, the dome-shaped sheet, and the horizontal sheet.

We videotaped the attack behavior on different prey types to determine the possible function of the different sections of the web. Each spider was offered a blow fly, a moth, a grasshopper, a damselfly, a bee (*Trigona* spp.), a katydid, or a leaf hopper every 2 days. Some prey items were placed directly on the lower, horizontal sheet of some webs. We made additional observations of the general shape of the web, prey captured, and attack behavior of spiders in the field. Voucher specimens of the spiders were deposited in the Museo de Zoología, Universidad de Costa Rica.

RESULTS

Webs of adult spiders.—Adult females of *T. sisyphoides* constructed their webs mostly on large, solitary individuals or small groups of *Agave* sp., *Yucca guatemalensis* (Agavaceae) and *Monstera deliciosa* (Araceae) plants scattered over the campus. These plants all have large, relatively rigid leaves. Other plants were seldom used. The webs consisted of a large tangle in which there was a dense, upper dome-shaped sheet; a more or less horizontal, much less dense sheet at the bottom; and a retreat at the top of the dome-shaped sheet in the midst of the tangle (Fig. 1). The dome-shaped and the horizontal sheets were very loosely connected at their borders, and there was an empty space without threads under the dome in which the spider moved freely during prey capture. The dense, irregular tangle above the dome-shaped sheet connected the dome to the substrates or to thick, multiple threads suspending the retreat. The border of the dome-shaped sheet was also connected to the substrates nearby (wire frame, or leaves and twigs in the field). The horizontal sheet was rarely connected to leaves or other substrates (3 out of 23).

Web construction.—*T. sisyphoides* (n = 15) began construction of the web between 1730 to 1830 h and ended the night's work at about 530 h next day (n = 5). Spiders took from one to four nights to construct a complete, functional web, although some additional threads were certainly added subsequently. The time spent in building decreased over successive nights. The first night the spiders were nearly continually active, spinning different parts of their webs, but on subsequent nights they began later (between 21 and 23 h), had longer pauses, and finished earlier (usually at 2 or 3 h). The spiders' only construction-oriented diurnal activity was to secure the retreat to the wire frame soon after the retreat was first placed in the wire frame.

Web construction can be roughly divided into five different stages, some of them not being mutually exclusive: exploration, suspension of the retreat, construction of the scaffolding, construction of the dome-shaped sheet, and construction of the lower horizontal sheet. The spider walked underneath silk lines at all times during construction. She held her dragline with the tarsus of one leg IV, frequently switching the leg IV that held the dragline.

Exploration: The spider began the construction of the web by exploring the wire cube. She climbed up to the frame along the threads that secured the retreat, then walked along the horizontal and vertical wires of the frame, attaching her dragline at irregular intervals and occasionally returning to the retreat. Sometimes the spider descended beyond the wire frame, from 30 cm to nearly one meter, hanging from her dragline, and then climbing back up the dragline to the frame. While ascending, the spider sometimes packed the slack dragline into a mass, and a small white mass was observed near the point where she reached the frame. More frequently she did not reel up the dragline, and attached a loop, or sagging threads to the wire. Occasionally the spider descended

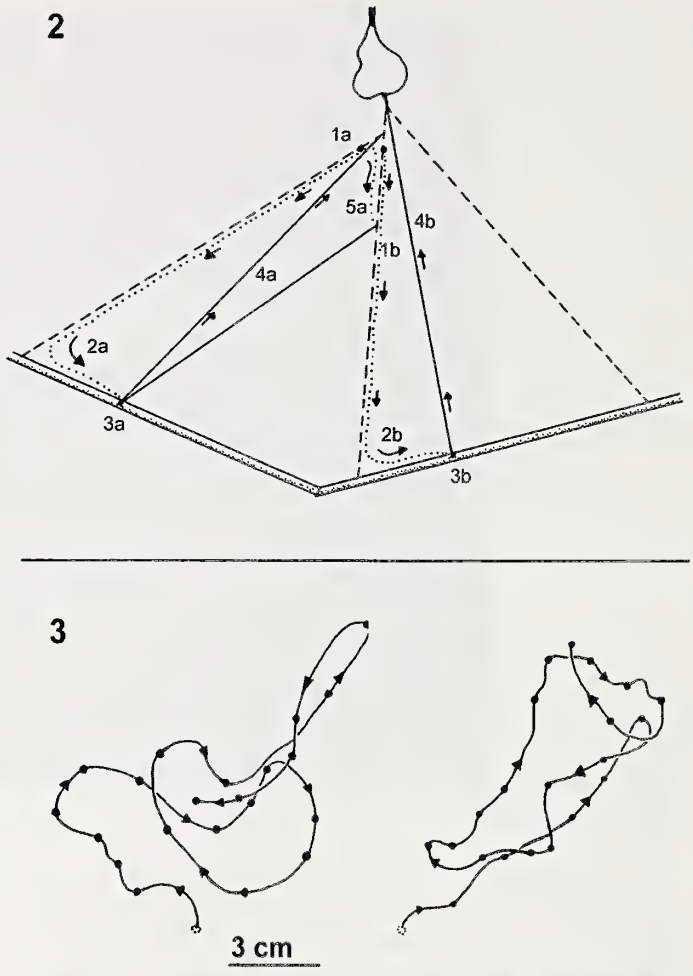
a second time. All spiders but one did not descend in this way from the frame during the exploration stage. The complete exploratory phase lasted 15–30 min.

Anchoring the retreat: After exploration, the spider began to reposition the retreat. First the spider walked up the line supporting the retreat and along a horizontal wire of the upper wire frame, away from the retreat, until she reached a vertical wire. The spider then descended a few centimeters along the vertical wire and attached the thread from the retreat to the wire. The spider often reinforced this line by walking back to the retreat on the same thread, doubling it. Some of these threads were attached to the wire frame above the retreat, but others were attached to the vertical wires either at the level of the retreat or a few centimeters below it. Then the spider broke threads attached to the upper section of the retreat, causing it to drop approximately 1 cm. This sequence was repeated several times until the retreat was moved up to nearly 10 cm downward from its original position, and was reoriented so that its opening was directed downward. The broken lines were occasionally packed. In these cases, the spider moved along another line while reeling up the cut line. She packed the loose line with her legs II and III, and then attached the whitish mass of silk to the wire frame or to another thread.

With the retreat in position, the spider began to spin threads from the top of the retreat to the upper wires, within the nearest 5 cm of the crisscrossing point of the diagonal wires. These threads were frequently reinforced by the spider walking back and forth, up to five times, on the same threads between the retreat and the furthest attaching point, forming thick cables that were clearly distinguishable from other threads. During construction of this cable, the spider was frequently observed attaching the new threads to those previously made. Construction of other sections of the web did not begin until the retreat was securely suspended from the upper section of the wire frame.

Construction of the scaffolding: Immediately after suspending the retreat, the spider began to construct the scaffolding for the dome-shaped sheet. She first spun threads that extended from the retreat opening, or near to it, to the wire frame. Additionally, she spun threads from some point along these threads to the wire frame, so that only five to six (n = 2 webs) threads converged at (or near) the retreat opening. These threads were then interconnected, forming a roughly conical scaffolding just below the retreat.

To spin the first threads of the conical scaffolding, the spider walked along one of the threads from which the retreat was suspended and then descended by one of the vertical wires. She then either attached her dragline to the vertical wire or continued to descend to the horizontal, bottom wire frame where she attached the dragline, touching her spinnerets repeatedly on the side of the wire facing the web or on the side away from the web. Once the thread was attached, the spider ascended by the thread she just had created and attached the new thread to it, producing a double line, or else she attached the new thread to another thread she encountered on the way up, usually a few centimeters away from the retreat opening. Only rarely, this second thread was attached at the retreat opening. After some lines were present below the hub, the spider descended by a previous thread, walked 2–4 cm along the wire, attached her dragline, and ascended by this new thread. This new thread was sometimes attached to the thread



Figures 2, 3.—Behavioral sequences during web construction. 2. Placement of threads during the scaffolding construction. The numbers (1a–5a and 1b–4b), arrows, and dotted lines mark the sequence and direction of movements of the spider during lines placement. Dashed lines indicate the pre-existing threads and solid lines indicate newly placed threads. 3. Two different paths of the spider as she filled in the dome-shaped sheet (100 s each traced from video images recorded looking approximately perpendicular to the plane of the sheet). Black dots indicate the position of the spider every 5 s.

she ascended, producing a double thread (Fig. 2: 1b–4b), or others to another thread (Fig. 2: 1a–5a).

When the spider had spun most lines (lines were difficult to observe and were not counted) forming the conical scaffolding, she connected these lines, and also connected them to the lines suspending the retreat. She also spun additional lines connecting the middle part of the retreat to pre-existing lines. The lines connecting the scaffolding of the dome-shaped sheet to the retreat suspension lines and to the upper section of the wire cube constituted part of the upper tangle. Video recordings showed that when attaching the dragline to another line, the spider held the dragline with one leg IV, while ipsilateral legs III and IV grasped the other line, bringing it toward the spinnerets at the same time that she bent her abdomen ventrally toward the line to touch it with her spinnerets.

Construction of the dome-shaped sheet, the horizontal sheet, and upper tangle: After the spider had constructed the scaffolding, she filled in the dome-shaped sheet. The process of filling in this sheet alternated with the construction of the

horizontal sheet and with the upper tangle. All spiders constructed the dome-shaped sheet in two phases: first the spider wove a complete but sparse dome-shaped sheet; then she filled in the spaces in this sheet. The sparse dome-shaped sheet was constructed in the first (n = 4) or second night (n = 11). During the second and third nights, the spiders increased the density of the dome-shaped sheet and of the threads of the upper tangle, which mostly consisted of threads connecting the dome-shaped sheet with the wires above. Construction of the horizontal sheet did not begin until the dome-shaped sheet had been partially built. The horizontal sheet was much less densely woven (Fig. 1).

The spider spent 1–3 min filling in a relatively small section of the dome-shaped sheet, then moved to a different section, sometimes on the opposite side of the dome, or sometimes nearby. After filling in a section, the spider often went up to the retreat, tapped the egg sac, then moved away to the next web section to fill in. The repeated visits to the retreat did not increase the number of threads converging at its mouth (n = 2 webs). During the filling in activity, the spider walked under the sheet rapidly forward, and sideways in an irregular pattern (Fig. 3), while tapping actively with both legs I. We did not see individual threads in all cases, but based on the spider's behavior in video analyses, the spider did not attach her dragline to all threads she came in contact with, since she walked several millimeters and frequently several centimeters without attaching her dragline. During the dragline attachments, the spider displayed two different movements: in one, she held the dragline with one leg IV, while ipsilateral legs III and IV grasped the sheet line, and brought it toward the spinnerets; in the other, the spider's two legs IV grasped the sheet simultaneously on either side of her spinnerets while her abdomen bent ventrally toward the lines and no leg held the dragline. We clearly observed both types of attachment behaviors in the construction of both the dome-shaped and the horizontal sheets.

Most spiders had constructed the scaffolding (14 out of 15) and part of the dome-shaped sheet by the end of the first night. Only four spiders constructed a complete web during the first night. By the end of the third night, all but one spider that never constructed the horizontal sheet had complete webs. All spiders added more threads, primarily to the dome-shaped sheet and to the tangle above it in subsequent nights. Filling in the dome-shaped sheet consumed most of the construction time of the spider (about 70%) on subsequent nights.

Viscid balls.—Viscid globules were present on threads of both the dome-shaped and the horizontal sheets in all webs examined (Fig. 4). Globules measured 58.5 ± 30.8 × 52.0 ± 29.3 µm (n = 20 globules, 5 webs) on dome-shaped sheet lines and 100.0 ± 62.0 × 88.3 ± 56.7 µm on lines in the horizontal sheet (n = 6 globules, 2 webs). Their mean density was lower in the horizontal sheet (0.94 balls/mm, SD = 0.62; 2 webs; 26 mm of thread sampled) than in the dome-shaped sheet (1.5 balls/mm, SD = 1.3; 4 webs; 22 mm of thread sampled). Globules were hydrophilic and increased in size in a humid-saturated environment.

Dissecting function of the web.—In nature, seven flies in at least three different families, five treehoppers (Membracidae), two beetles (one Scarabaeidae, one Chrysomelidae) and one honey bee were found in webs (n= 22). Most prey that were dropped on webs were retained for several seconds in the upper

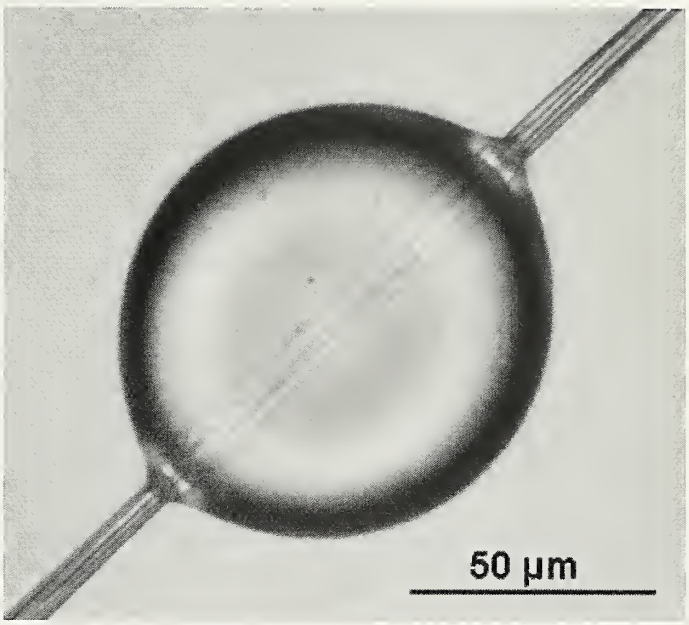


Figure 4.—Viscid globule from the dome-shaped sheet of *Tidarren sisyphoides*. The globule is on a pair of core fibers.

tangle (27 out of 30) before dropping to the dome-shaped sheet. The spider sensed the prey as soon as it contacted the upper tangle, first orienting inside her retreat (this was not possible to observe in all cases) and then moving to the area of the dome sheet beneath the struggling prey. There she pulled some threads, turned a few degrees, and pulled other threads until the prey contacted the dome-shaped sheet. On one occasion, the spider broke the threads of the dome-shaped sheet and climbed up to attack the prey in the upper tangle. Prey that fell to the dome-shaped sheet were generally constrained until the spider arrived, but in a few cases, large, strong, struggling insects (e.g., katydids) broke free from the upper tangle and dome-shaped sheet. These prey hit the horizontal sheet, but were not trapped there long enough for the spider's attack. Prey that were placed directly on the upper face of the horizontal sheet (n = 7) were not restrained long enough to allow the spider attack the prey.

Attack behavior.—Attacks began by applying viscid threads on to the prey with both simultaneous and alternate movements of legs IV. If the prey was dangerous (e.g., katydids), viscid threads were applied from farther away than to flies or moths. Wrapping continued until prey was immobilized, at which point it was bitten. In 83% of 72 spider-prey encounters, the spider retired to her retreat and returned to the prey after the prey's movements had subsided. When the prey was large, the spider cut it free from the dome-shaped sheet before continuing the wrapping attack as it hung on a few lines below the level of the dome. If prey's movements had not completely subsided when the spider returned from her retreat, the prey was wrapped and bitten again. Then it was carried, dangling from one leg IV to near the retreat where it was wrapped some more and attached to the threads near the mouth of the retreat.

DISCUSSION

The complex aerial-sheet web of *T. sisyphoides* seems to be unusual in several respects among theridiids. Several other

theridiids (e.g., *Anelosimus* spp., *Chrosiothes portalensis*, *Achaearanea tesselata*, *A. disparata*, *A. japonica*) also construct webs with horizontal or bowl shaped aerial-sheets (Darchen 1968; Eberhard 1972; Darchen & Ledoux 1978; Eberhard et al. 2008a), but never a dome-shaped sheet as in *T. sisyphoides* (and also some webs of *T. haemorrhoidale* Eberhard et al. 2008a). The aerial sheets have most likely evolved independently in these theridiid lineages, as indicated by a recent phylogenetic study that showed an extremely high flexibility in web-building behaviors and high convergence in web features among theridiids (Eberhard et al. 2008a). However, the dome-shaped sheet and the presence of a horizontal sheet connected to the border of the dome-shaped sheet (Fig. 1) seem to be unique features of the *Tidarren* genus; a horizontal sheet connected to the dome-shaped sheet has only been found in *T. sisyphoides*. The absence of similar elements in webs of other theridiid species (Agnarsson 2004; Eberhard et al. 2008a) suggests that, at least, some elements of the *T. sisyphoides*' web represent an independent and unique event in the evolution of webs in Theridiidae.

Despite the unusual design of the webs of *T. sisyphoides*, there are several general behavioral patterns in the web construction that resemble those behaviors of other theridiid species that have quite different webs. *T. sisyphoides* explored prior to initiating web construction, constructed its web only at night, constructed a scaffold that supports the rest of the web, alternated construction of different sections of the web, held its dragline with one leg IV, doubled lines during the scaffold construction, and added new threads and repaired the web over many subsequent nights. These behavior patterns are similar to those of other species of Theridiidae: *Latrodectus*, *Steatoda*, *Theridion*, and *Achaearaea* (Szlep 1965; Eberhard 1982; Benjamin & Zschokke 2002, 2003; Jörger & Eberhard 2006; Barrantes & Weng 2007; Eberhard et al. 2008b), indicating that they are widespread within theridiids. Similar behaviors occur in other spider families. For instance, holding the dragline with one leg IV, alternating construction of different parts of the web, and doubling threads has also been described for other Orbiculariae (Eberhard 1990b). Descending from the retreat (or near to it) along a pre-existing thread while putting out a new line, walking on the substrate, attaching this new line to the substrate, and then ascending by this new line to return to the retreat (or near to it) during the scaffolding construction is another behavior that has also been described for *Steatoda triangulosa* and *A. tepidariorum* (Benjamin & Zschokke 2002, 2003). This order of thread placement is similar to, though less stereotypical of, radius construction in the Nephilidae and Uloboridae (Eberhard 1982; Kuntner et al. 2008). However, further phylogenetic based studies are necessary to determine whether these behaviors are homologous between Theridiidae and other Orbiculariae.

The detailed description of web-construction by *A. tesselata* (Jörger & Eberhard 2006), allows us to further compare the construction behavior between this species and *T. sisyphoides*. Both species strengthened the lines holding up the retreat prior to initiating construction of the web. Securing the retreat first is likely due to the fact that in both species the spiders that were observed had either egg sacs or spiderlings in their retreats; in nature, these spiders first construct a web, and then collect a curled leaf or other plant debris to construct the retreat. Attaching the anchor and scaffolding lines to the far side of objects that likely make attachments more secure, occurs also in *A. tesselata* and some orb-weaver araneoids (Jörger & Eberhard 2006; Eberhard 1990b; Eberhard 2001). Breaking and releasing threads is frequent during some phases of the web construction of these two species, as well as in *S. triangulosa* (Benjamin & Zschokke 2002) and *L. geometricus* (Eberhard et al. 2008b). This behavior may be at least partially explained by an inability of theridiids to digest silk, but it is also possible that loose threads might increase prey retention in the web (Kirchner 1986; Blackledge et al. 2008). Break and reel behavior was not observed in *T. sisyphoides*, though it occurs in *A. tesselata* (Jörger & Eberhard 2006) during exploration, and in *A. tepidariorum* (W. Eberhard pers. comm.), and *L. geometricus* during gum foot line construction (Eberhard et al. 2008b).

Sheet construction by *T. sisyphoides* also resembled that of *A. tesselata*. The spider walked under silk lines while constructing the sheet, filling in different parts of the sheet in no apparent order (perhaps more detailed observations might establish some pattern). Attachments of the dragline were similar in both species: the spider used either one or both legs IV to hold sheet threads when she attached her dragline during filling in behavior. Both species filled in the sheet with apparently erratic wandering movements, although they were apparently more regular in *T. sisyphoides*. Both species often returned to the retreat during filling-in behavior, presumably using lines previously laid in the near vicinity of the retreat. This behavior results in only a few lines converging at the mouth of the retreat, and explains the ability of the spider to orient inside the retreat toward the prey in the web before launching an attack (Barrantes & Weng 2006a; Jörger & Eberhard 2006). Having few threads converging at the retreat is also a feature of newly constructed webs of several *Latrodectus* (Szlep 1965; Eberhard et al. 2008b).

Some general behaviors (e.g., construction of scaffolding, expansion of web over time) are widely spread within theridiids. However, some other traits such as the presence of an aerial sheet in the web have probably evolved independently several times within Theridiidae (Jörger & Eberhard 2006; Agnarsson 2004; Eberhard et al. 2008a), and other families (e.g., Linyphiidae, Pholcidae, and Synotaxidae-*Chileotaxus sans*) possibly as a result of using similar habitats, capturing similar prey types (Wise 1982), and predation and parasitism pressure (Blackledge et al. 2003; Agnarsson 2004).

The dome-shaped sheet and the tangle above it (upper tangle) seem to function as the trapping section of the web. The upper tangle probably functions to knock down jumping and flying insects that are then restrained by viscid elements in the dome-shaped sheet, as indicated by the insect types found in nature. The horizontal sheet at the bottom of the web seems to have little effect in prey retention; perhaps it serves as a barrier to reduce attacks of predators and parasitoids (Lubin 1986; Blackledge et al. 2003). Viscid balls have not previously been reported in webs of species in this genus (Benjamin & Zschokke 2003; Agnarsson 2004; Eberhard et al. 2008a). The viscid balls of gum foot lines and the viscous wrapping silk in theridiids are apparently produced by the aggregate glands (Kovoor 1977; Coddington 1989). However, until the origin of the core axial fiber on which *T. sisyphoides* place the viscid balls is clearly established, it will be possible to determine

whether these viscid threads are homologous to the gum foot lines or other sticky threads of other theridiid webs (Eberhard et al. 2008a).

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Scorpion taphonomy: criteria for distinguishing fossil scorpion molts and carcasses

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Abstract. The ability to distinguish fossil arthropod carcasses from their molts is necessary for a more complete understanding of the arthropod fossil record and for more accurately assessing the role of fossil arthropods in paleoecosystems. Taphonomic characteristics, e.g., recurrent patterns of disarticulation of exoskeletal elements, are the primary data that have been used to differentiate fossil exuvia and fossil carcasses among arthropods. This study documents recurrent taphonomic patterns in modern scorpion carcasses and molts and extends these patterns to the fossil record to define criteria by which fossil scorpion molts might be distinguished from fossil scorpion carcasses. The three most useful and statistically significant characters in making the scorpion carcass/molt distinction are: position of the chelicerae (drawn in or extended); position of walking legs (folded or splayed); and body line (straight or curved). Two other characteristics, the position of pedipalps and presence or absence of telescoped segments, approach statistical significance and are also potentially useful. Disarticulation data are not as useful for distinguishing fossil scorpion molts and carcasses, because there are no statistically significant differences in length of time to total disarticulation or in the sequence of disarticulation between scorpion molts and carcasses. Among extant arthropods, scorpions possess the body plan most similar to that of the extinct eurypterids. Therefore, the taphonomic criteria developed for distinguishing fossil scorpion molts and carcasses may have implications for understanding molting among eurypterids.

Keywords: Arthropod, ecdysis, eurypterid

Scorpions are terrestrial chelicerates that have a fossil record extending back more than 400 million years to the Silurian Period. Fossil scorpions, although relatively rare, can be well preserved (Menon 2006). However, the relative importance of fossil scorpions in ancient ecosystems is difficult to assess because of the arthropod trait of growth through ecdysis. An individual scorpion may accumulate a number of molts over its lifetime; these molts may not be distinguished easily from carcasses, especially after becoming fossils, thereby complicating interpretation of the scorpion fossil record. This is also the case with fossil horseshoe crabs (Chelicerata: Xiphosura), whose fossil carcasses and molts are often indistinguishable (Babcock et al. 2000). Therefore, for many fossil arthropod taxa, simple or apparent abundance may not be a reliable reflection of actual abundance. Without accurate abundance estimates, it is difficult to determine the importance of these arthropods in paleoecosystems.

There are a number of differences between modern scorpion molts and carcasses, such as the presence of internal organs in carcasses and the lack of most of these structures in exuvia, but differences based on internal anatomy are not readily apparent in fossils; indeed, internal structures usually cannot be discerned in fossil scorpions (but see: Wills 1925, 1946, 1960; Kjellesvig-Waering 1986). External features of the exoskeleton, such as the presence or absence of appendages, opened sutures, and dislocations (separations) between segments, are more readily accessible characters for making the potential distinction between carcasses and exuvia among fossil arthropods. These kinds of taphonomic observations of the scorpion exoskeleton are the focus of this investigation. The purpose of this paper is twofold: 1) to document whether different taphonomic patterns exist between modern scorpion carcasses and molts; and 2) to use these patterns, if present, to develop criteria for distinguishing fossil scorpion molts from carcasses.

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PREVIOUS WORK ON SCORPION MOLTING AND TAPHONOMY

Scorpion molting is well understood (Polis 1990; Brownell & Polis 2001; Gaban & Farley 2002). The steps scorpions follow during molting do not appear to vary significantly among taxa (Rosin & Shulov 1962; De Armas 1986): the anterior suture opens and the animal moves forward to exit the old exoskeleton, in a manner similar to that of a horseshoe crab (Shuster 1982). A scorpion exuvium may include the booklung lamellae, preoral tube, and other internal features and there may be little distortion of delicate hairs, bristles, and setae (Gaban & Farley 2002). The exuvium is commonly intact except for a wedge-shaped gap beneath the carapace (Gaban & Farley 2002). These observations on modern scorpion exuvia suggest that, as in the case of horseshoe crabs, a well-preserved fossil scorpion exuvium may be difficult to distinguish from a well-preserved fossil scorpion carcass. In this study, we identify more readily observed external features of the scorpion exoskeleton as the basis for distinguishing scorpion molts and carcasses.

METHODS

Six species of living scorpions belonging to six genera were used in the taphonomy experiments (Table 1). These taxa were chosen because they were available in sufficient quantity for replicate experiments. The scorpion molts used in this study were donated by arachnid hobbyists; live scorpions were purchased through a commercial supplier (www.swiftinverts.com) or were donated by arachnid hobbyists. Scorpion carcasses were obtained through either the natural death of the animal or mortality through freezing. We collected two categories of taphonomic data on scorpion molts and carcasses: 1) the initial post-mortem (for carcasses) or post-

Table 1.—Taphonomic characteristics of scorpion molts and carcasses. Significant p-values are in bold. [1]This cell does not total 13 because six exuvia lack appendages. [2]Chi-square with Yates correction, all one degree of freedom.

Character	Description	Molt (n = 13)	Carcass (n = 13)	Chi-square[2]	P
Chelicerae	Extended	12	0	18.73	**1.5×10^{-5}**
	Retracted	1	13		
Pedipalps	Extended	11	6	2.71	0.10
	Retracted	2	7		
Body line	Curved	8	0	8.85	**0.003**
	Straight	5	13		
Walking legs	Splayed	7[1]	3	7.91	**0.005**
	Folded	0	10		
Telescoping of segments	Present	4	0	2.66	0.10
	Absent	9	13		

molt (for exuvia) exoskeletal posture and 2) the order and timing of disarticulation of the exoskeleton (i.e., the disarticulation sequence) through subsequent tumbling experiments.

Death/molt posture.—Intact scorpion carcasses and exuvia were photographed, and the following exoskeletal characteristics were recorded for each specimen: 1) curvature of the mesosoma and metasoma; 2) presence or absence of telescoped mesosomal and metasomal segments; 3) position/orientation of the chelicerae; 4) position/orientation of the walking legs; and 5) position/orientation of the pedipalps. A total of 13 carcasses and 13 molts were used in this study. Twenty-four fossil scorpions from the collections of the Yale Peabody Museum (YPM) were photographed and described following the same five criteria.

Tumbling.—After documenting the initial condition of the exoskeleton, scorpions were treated in one of three ways: 1) tumbling (wet)–13 carcasses and 11 exuvia were tumbled to the point of complete disarticulation in fresh water inoculated by the addition of water from an aquarium; 2) tumbling (dry)--one carcass and two exuvia were tumbled dry until disarticulated; 3) one carcass and one exuvium were left to decay in inoculated freshwater with no agitation as controls. Wet tumbling was done in one of two small (5-cm radius) plastic rock-tumblers available from hobby stores. One tumbler barrel had a smooth interior; the other tumbler had molded invaginations of the barrel wall that acted as interior bails. Dry tumbling of larger specimens was done in a larger (20-cm radius) tumbler with two internal bails. The tumblers were checked daily and the extent of disarticulation of the specimens recorded. Carcasses were kept frozen until used in the tumbling experiments to minimize potential differences in disarticulation between specimens due to differences in the degree of decay (Allison 1986).

Table 2.—Laboratory treatment of scorpion carcasses and molts.

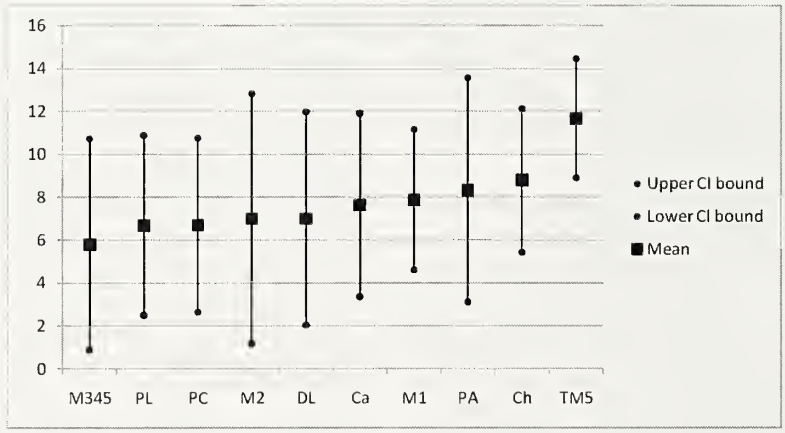
Species	Molt/Carcass	Wet/Dry	Tumbler	Time (days)
Pandinus imperator (C.L. Koch 1841)	molt	dry	bail-lg	2
Leiurus quinquestriatus (Ehrenberg 1928)	molt	dry	smooth	16
P. imperator	molt	wet	smooth	14
P. imperator	molt	wet	smooth	7
Parabuthus transvaalicus	molt	wet	smooth	15
P. transvaalicus	molt	wet	smooth	11
P. imperator	molt	wet	smooth	18
P. imperator	molt	wet	smooth	12
P. imperator	molt	wet	bail	18
P. imperator (juv)	molt	wet	smooth	10
P. imperator	molt	wet	smooth	11
P. imperator	molt	wet	bail	6
P: imperator (juv)	molt	wet	smooth	7
P. imperator (juv)	carcass	dry	bail-lg	4
P. imperator (juv)	carcass	wet	smooth	22
Anuroctonus phaiodactylus (Wood 1863)	carcass	wet	smooth	8
Smeringurus mesaensis (Stanhke 1957)	carcass	wet	smooth	19
S.mesaensis	carcass	wet	bail	18
S.mesaensis	carcass	wet	smooth	11
S.mesaensis	carcass	wet	bail	10
S.mesaensis (juv)	carcass	wet	bail	6
S.mesaensis	carcass	wet	smooth	13
S.mesaensis	carcass	wet	bail	10
S.mesaensis	carcass	wet	smooth	11
S.mesaensis (juv)	carcass	wet	bail	3
S.mesaensis (juv)	carcass	wet	smooth	13
Vaejovis spinigerus (Wood 1863)	carcass	wet	smooth	12

Table 3.—Comparison of molt/carcass and tumbler effects on disarticulation. *P*-values from the *t*-tests on the mean time to separation of exoskeletal tergites; significant *P*-values are in bold. S = smooth tumbler, B = tumbler with bails, PC = pedipalp claws, PA = pedipalp appendages, DL = distal leg segments, PL = proximal leg segments, M345 = third, fourth and fifth metasomal segments as one unit, M2 = second metasomal segment, M1 = first metasomal segment, Ch = chelicerae, Ca = carapace, and TMS = total mesosomal separation (= total exoskeletal disarticulation). The lack of significant *P*-values in the first row indicates that there are no differences in the timing of disarticulation between molts and carcasses. In the second row, the majority of the *P*-values are significant, reflecting differences in the timing of disarticulation between specimens in the smooth tumbler and specimens in the tumbler with the bail.

	PC	PA	DL	PL	M345	M2	M1	Ch	Ca	TMS
molt vs carcass	0.6817	0.7332	0.9333	0.9858	0.65	0.932	0.605	0.4175	0.363	0.891
S- vs. B-tumbler	**0.0001**	**0.0001**	**0.0083**	**0.0085**	**0.005**	**0.0025**	0.939	**0.0053**	0.089	0.255

Statistics.—Standard chi-square tests of independence with Yates correction (Preacher 2001) were used to compare the frequency of each of the five exoskeletal characteristics of death/molt postures listed above. To adjust for multiple tests, we modified the standard alpha level of 0.05 as per the conservative Bonferroni correction by dividing by 5 (the number of characteristics tested), so that *P*-values less than 0.01 were considered statistically significant (Preacher 2001). The chi-square calculations were carried out on an online calculator (Preacher 2001).

We used *t*-tests to compare the mean time-to-separation of various exoskeletal elements: pedipalp claws, pedipalp appendages (tibia and brachium), distal leg segments, proximal leg segments, last three metasomal segments, second metasomal segment, first metasomal segment, chelicerae, carapace, and total mesosomal separation. Two variables were considered: specimen type (molt or carcass) and tumbler type (smooth interior/bails). *P*-values less than 0.05 were considered significant. The sequence of disarticulation suggested by the mean time-to-separation of each



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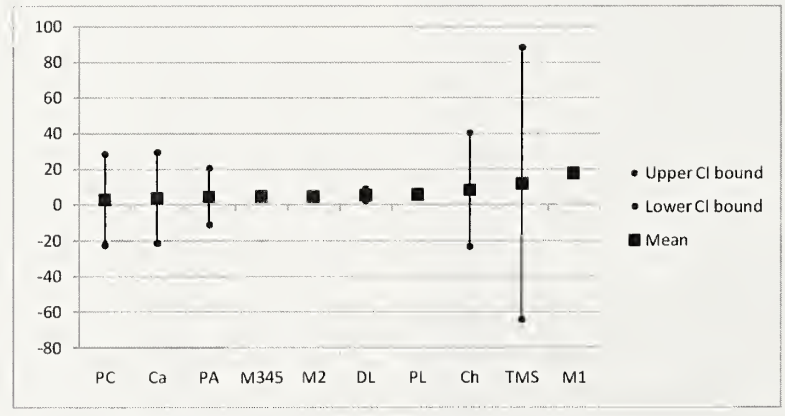

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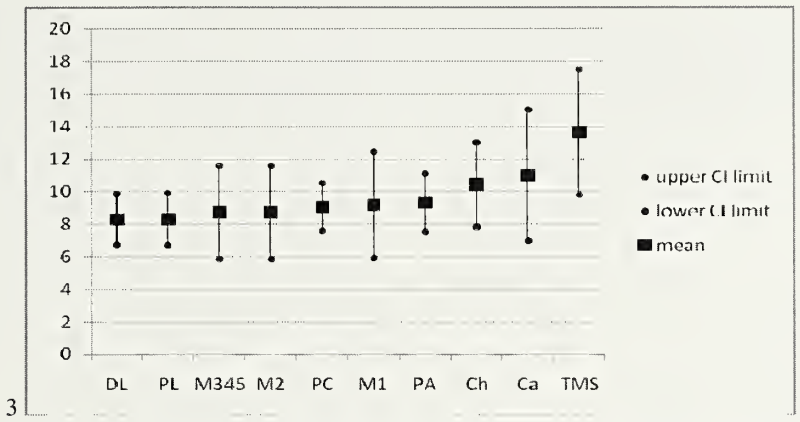
Figures 1, 2.—Plots of mean time to separation data showing order and timing of disarticulation for scorpion molts under different tumbling conditions. The y-axis is days. The vertical bars represent 95% confidence intervals. A small sample size results in a large standard deviation, which explains 'negative days' in Figure 2. The bars are ordered according to increasing mean time to separation (the center of each bar); the arms of the bars show the entire expected range of time to separation: 1. molts tumbled in smooth canister $n = 9$; 2. molts tumbled with agitation (bail), $n = 2$; PC = pedipalp claws, PA = pedipalp appendages, DL = distal leg segments, PL = proximal leg segments, M345 = third, fourth and fifth metasomal segments as a single unit, M2 = second metasomal segment, M1 = first metasomal segment, Ch = chelicerae, Ca = carapace, and TMS = total mesosomal separation (= total exoskeletal disarticulation).



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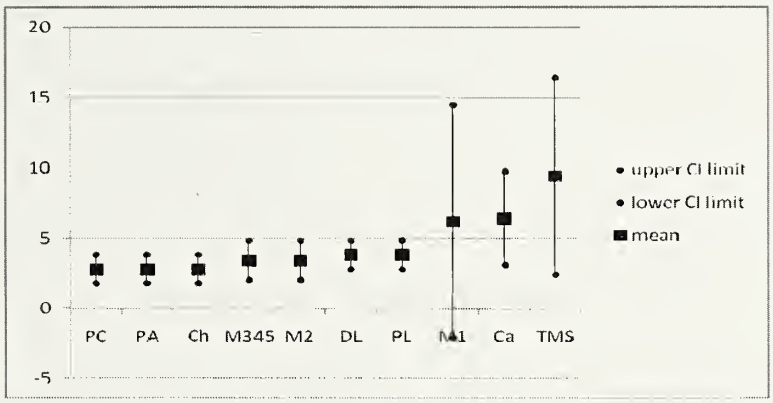

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Figures 3, 4.—Plots of mean time to separation data showing order and timing of disarticulation for scorpion carcasses under different tumbling conditions. The y-axis is days. The vertical bars represent 95% confidence intervals. The bars are ordered according to increasing mean time to separation (the center of each bar), the arms of the bars show the entire expected range of time to separation: 3. carcasses tumbled in smooth canister, $n = 8$; 4. carcasses tumbled with agitation, $n = 5$. PC = pedipalp claws, PA = pedipalp appendages, DL = distal leg segments, PL = proximal leg segments, M345 = third, fourth and fifth metasomal segments as a single unit, M2 = second metasomal segment, M1 = first metasomal segment, Ch = chelicerae, Ca = carapace, and TMS = total mesosomal separation (= total exoskeletal disarticulation).

exoskeletal element was also tested using 95% confidence intervals.

The *t*-tests and 95% confidence intervals were generated using SAS software, version 9.2 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA).

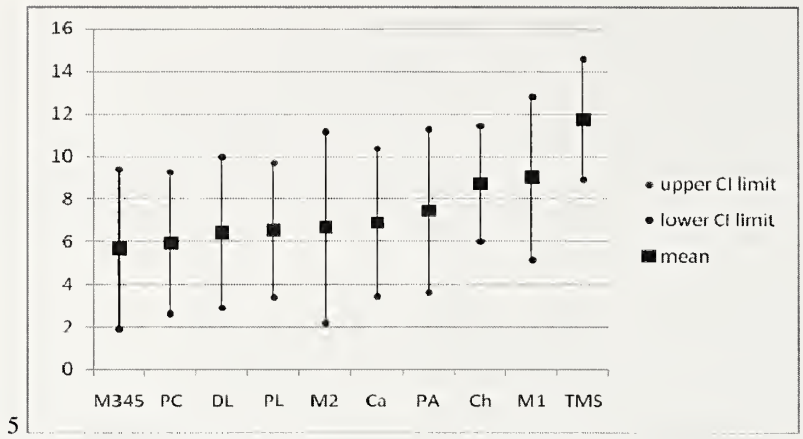
RESULTS

Taphonomic patterns, I. Death/molt posture.—The scorpion carcasses examined for this study exhibited the following characters: 1) retracted chelicerae (100% of the specimens); 2) straight body line (in dorsal view) with the metasoma extended straight back (100%); 3) pedipalps pulled in toward the prosoma (54%); and 4) walking legs folded against the body (77%) (Table 1 and Figures 7–18). In contrast, molts were characterized by the following features: 1) extended chelicerae (92%); 2) curved body line and curved metasoma (62%); 3) pedipalps pulled well back from the prosoma in an extended position (85%); and 4) splayed walking legs (100%) (Table 1 and Figures 7–18). Four of 13 molts (31%) exhibited telescoped mesosomal segments and overlap of the ventral surface on the dorsomedial surface (Figures 11, 15). These observations held across the six different scorpion genera examined for this study, suggesting taxonomic independence of these body-posture criteria. The low *P*-values obtained in the chi-squared test (Table 1) indicate that our sample size is sufficiently large to reflect a significant difference ($P < 0.05$) between scorpion death and molt postures.

Taphonomic pattens, II. Disarticulation.—Previous actuotaphonomic studies on tumbling segmented, exoskeleton-bearing invertebrates suggested that freezing the animals does not affect the exoskeletal disarticulation sequence (Kidwell & Baumiller 1990 on echinoids). We detected no differences in disarticulation between scorpions that had been frozen versus scorpions that had died naturally. Time-to-total disarticulation of the specimens is given in Table 2.

Wet vs. Dry: Both exuvia and carcasses released complete, intact tergites under wet tumbling conditions. These elements were not broken by subsequent tumbling (tumbling was terminated with the dissociation of the last exoskeletal elements). Carcasses tumbled dry behaved as carcasses tumbled wet. However, molts tumbled dry were quickly (2–3 days) reduced to ragged-edged, broken exoskeletal pieces rather than separated, unbroken tergites.

Disarticulation Sequence: A disarticulation sequence comprises two components: order and timing. Order refers to the sequence in which a given exoskeletal element separates in relation to the other exoskeletal elements. For example, "legs, pedipalps, chelicerae" is a different disarticulation order than



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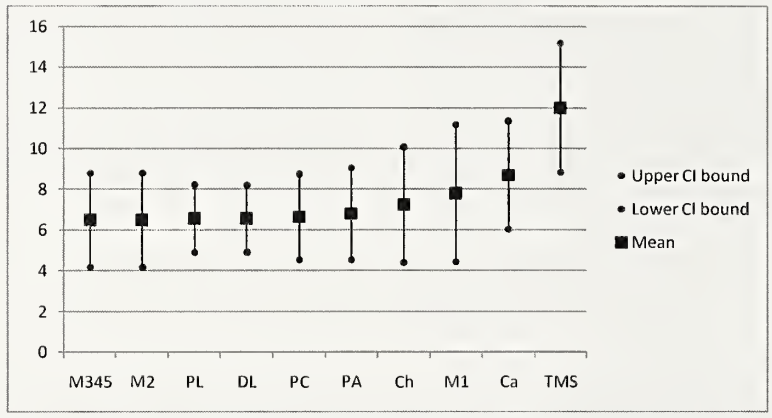

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Figures 5, 6.—Plots of mean time to separation data showing order and timing of disarticulation for all scorpion molts and carcasses. The y-axis is days. The vertical bars represent 95% confidence intervals. The bars are ordered according to the mean time-to-separation (the center of each bar); the arms of the bars show the entire expected range of time to separation: 5. all molts, $n = 11$; 6. all carcasses, $n = 13$. PC = pedipalp claws, PA = pedipalp appendages, DL = distal leg segments, PL = proximal leg segments, M345 = third, fourth and fifth metasomal segments as a single unit, M2 = second metasomal segment, M1= first metasomal segment, Ch = chelicerae, Ca = carapace, and TMS = total mesosomal separation (= total exoskeletal disarticulation).

"pedipalps, legs, chelicerae." Timing refers to the elapsed time before an exoskeletal element separates. Only differences in order are noticeable in fossils, but a difference in order necessarily requires a difference in timing.

Molts and carcasses showed no significant differences in mean time to separation in any of the exoskeletal elements (Table 3, Row 1), indicating that there is no significant difference in the sequence or order of disarticulation for scorpion molts as compared to scorpion carcasses. Plots of 95% confidence intervals of the mean time to separation for each exoskeletal element (Figures 1–6) show a general pattern shared by molts and carcasses. The appendages (PC, PA, DL, PL, Ch in Figures 1–6) and metasoma (M345, M2, M1 in Figures 1–6) tend to separate from the rest of the body before the mesosoma disarticulates (TMS in Figures 1–6). For example, in Figure 6, the pedipalps separate on day 6, and the mesosoma disarticulates on day 12.

Using the rock-tumbler with the internal bails shortened disarticulation time for both molts (compare Figures 1 and 2) and carcasses (compare Figures 3 and 4). This tumbler effect is also reflected in the statistically significant values in Table 3, Row 2. However, the tumbler effect does not result in a statistically significant difference in disarticulation timing between molts and carcasses, as shown by the overlapping confidence intervals (compare Figure 1 with Figure 3 and Figure 2 with Figure 4).

A somewhat unexpected result was the comparison of times to total disarticulation. Molts proved to be as durable as carcasses when tumbled in water, as evidenced by the fact that the mean time to total disarticulation (see Table 3, Row 1, Column TMS, and Figures 5, 6) is not significantly different between molts and carcasses.

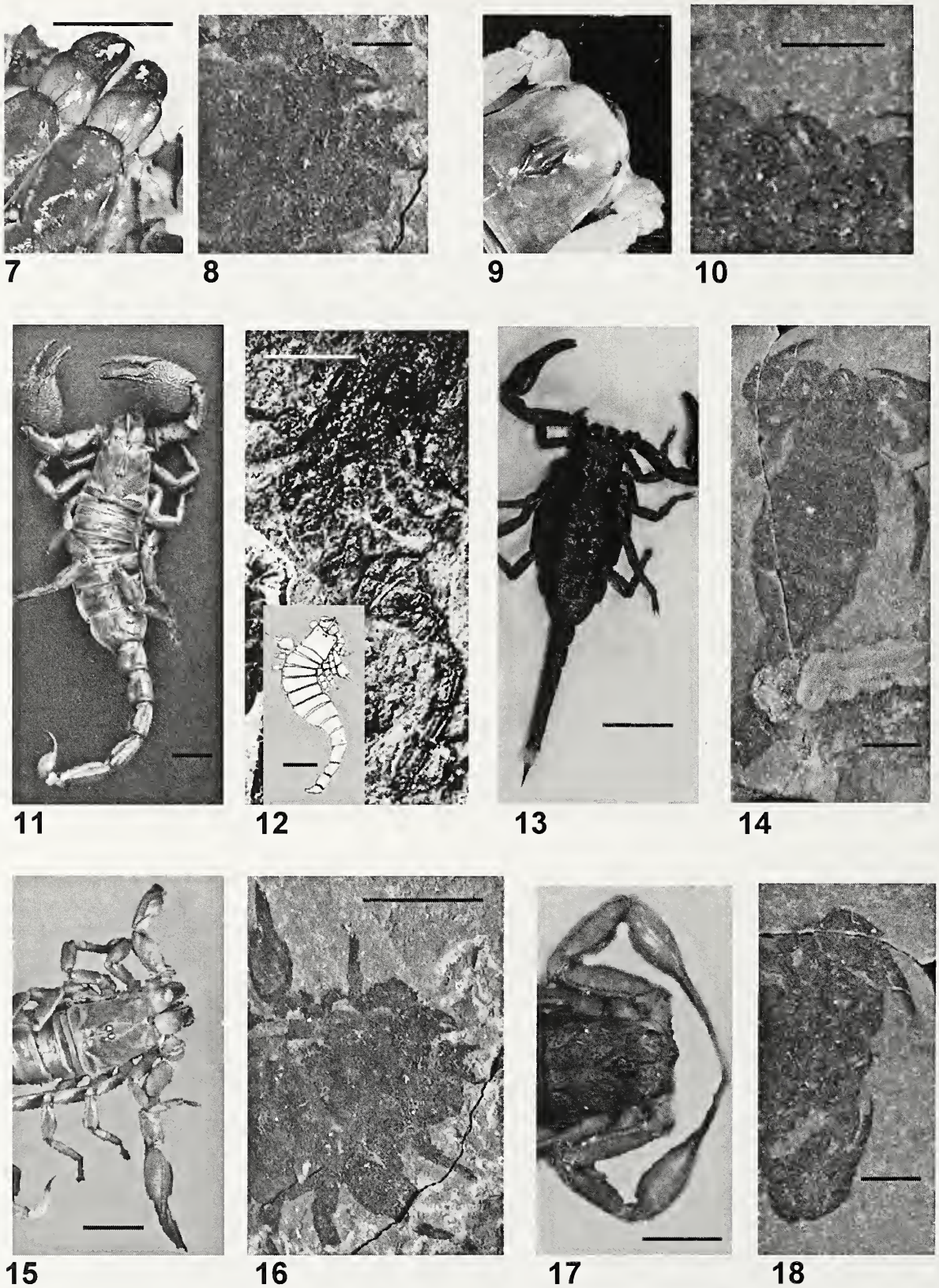
To summarize the statistical analysis of the tumbling data, molts and carcasses generally release appendages and metasomal segments early, the mesosoma is the most resilient exoskeletal unit for both exuvia and carcasses, and molts take as long as carcasses to reach total disarticulation.

DISCUSSION AND APPLICATION TO FOSSIL SCORPIONS

Scorpion molting and exuvia taphonomy.—Observations on ecdysis in modern scorpions support the criteria defined here of appendage position and body posture as diagnostic for distinguishing carcasses and exuvia. De Armas (1986) concluded that the position of the legs on a suitable substrate is critical for scorpions to successfully complete the process of ecdysis,

Table 4.—YPM specimens and identification as molt or carcass based on the criteria defined herein; "+"indicates presence, "-" denotes missing features or that determination could not be made, "?" denotes identification could not be determined due to poor preservation. E = extended, R = retracted, C = curved, St = straight, Sp = splayed, F = folded, P = present, A = absent. In the age column, MP = Middle Pennsylvanian, LS = Late Silurian.

		Chelicerae		Pedipalps		Body Line		Walking legs		Telescoping					
Specimen #	Taxon	E	R	E	R	C	St	Sp	F	P	A	ID	Locality	Age	Formation
typical carcass		-	+	-	+	-	+	-	+	-	+	carcass			
typical molt		+	-	+	-	+	-	+	-	+	-	molt			
YPM 126	*Anthracoscorpio? sp.*	-	-	-	-	-	+	-	-	-	+	?	Grundy Co., IL	MP	Carbondale
YPM 206691	*'Archaeophonus eurypteroides'*	+	-	+	-	-	+	+	-	-	-	molt	Herkimer, NY	LS	Willimsville
YPM 00128	*Eoscorpius carbonarius*	+	-	+	-	-	+	+	-	-	-	molt	Grundy Co., IL	MP	Carbondale
YPM 139	*E. carbonarius*	-	-	-	-	-	-	-	-	-	-	?	Grundy Co., IL	MP	Carbondale
YPM 138	*E. danielsi*	-	-	+	-	-	-	+	-	-	-	molt	Grundy Co., IL	MP	Carbondale
YPM 212155	*Mazonia woodiana*	+	-	+	-	-	+	-	-	-	-	molt	Welland, ON	LS	Fiddler's Green
YPM 127	*Palaeobuthus distinctus*	-	-	-	-	-	+	-	-	-	-	?	Grundy Co., IL	MP	Carbondale
YPM 212157	*P. distinctus*	-	-	-	-	+	-	-	-	-	-	molt	Grundy Co., IL	MP	Carbondale
YPM 140	*Palaeopisthacanthus schucherti*	+	-	+	-	-	-	-	-	-	-	molt	Grundy Co., IL	MP	Carbondale
YPM 130	*Proscorpius distinctus*	+	-	+	-	-	+	+	-	-	-	molt	Grundy Co., IL	MP	Carbondale
YPM 133	*P. distinctus*	-	-	+	-	+	-	-	-	-	-	molt	Grundy Co., IL	MP	Carbondale
YPM 208121	*P. osborni*	-	-	+	-	+	-	+	-	+	-	molt	Herkimer, NY	LS	Williamsville
YPM 208125	*P. osborni*	+	-	+	-	-	+	+	-	-	+	molt	Herkimer, NY	LS	Williamsville
YPM 208126	*P. osborni*	-	-	+	-	+	-	+	-	-	-	molt	Herkimer, NY	LS	Williamsville
YPM 208127	*P. osborni*	-	-	-	-	-	+	-	-	+	-	?	Herkimer, NY	LS	Fiddler's Green
YPM 208129	*P. osborni*	-	+	-	+	-	+	-	+	-	+	carcass	Herkimer, NY	LS	Williamsville
YPM 208130	*P. osborni*	+	-	+	-	+	-	+	-	-	-	molt	Herkimer, NY	LS	Williamsville
YPM 208131	*P. osborni*	-	-	+	-	-	+	-	-	-	+	carcass	Herkimer, NY	LS	Williamsville
YPM 209823	*P. osborni*	+	-	+	-	+	-	+	-	-	-	molt	Herkimer, NY	LS	Williamsville
YPM 212926	*P. osborni*	-	-	+	-	-	+	+	-	-	-	molt	Herkimer, NY	LS	Fiddler's Green
YPM 212927	*P. osborni*	-	-	-	-	-	+	-	-	-	-	?	Herkimer, NY	LS	Fiddler's Green
YPM 212928	*P. osborni*	-	-	-	-	-	-	-	-	-	-	?	Herkimer, NY	LS	Fiddler's Green
YPM 206692	*'Stoermeroscorpio delicatus'*	-	-	+	-	+	-	+	-	-	-	molt	Herkimer, NY	LS	Williamsville



Figures 7–18.—Comparison of fossil scorpion molts and carcasses: 7. Modern scorpion molt with extended chelicerae, scale bar is 5 mm; 8. Fossil scorpion molt with extended chelicerae, scale bar is 5 mm; 9. Modern scorpion carcass with retracted chelicerae, scale bar is 5 mm; 10. fossil scorpion carcass with retracted chelicerae, scale bar is 10 mm; 11. Modern scorpion molt with highly curved body line, scale bar is 10 mm; 12. Fossil scorpion molt with highly curved body line, each scale bar is 5 mm, from *Treatise on Invertebrate Paleontology,* ©1956, courtesy of the Geological Society of America and the University of Kansas; 13. Modern scorpion carcass with straight body line, scale bar is 10 mm; 14. Fossil scorpion carcass with straight body line, scale bar is 10 mm; 15. Modern scorpion molt with extended pedipalps, scale bar is 10 mm; 16. Fossil scorpion molt with extended pedipalps, scale bar 10 mm; 17. Modern scorpion carcass with retracted pedipalps, scale bar 10 mm; 18. Fossil scorpion carcass with retracted pedipalps, scale bar is 5 mm.

Table 5.—Literature survey with identification of illustrated specimens as molt or carcass based on the criteria defined herein; "+" indicates presence, "-" denotes missing features or poor preservation. E = extended, R = retracted, C = curved, St = straight, Sp = splayed, F = folded, P = present, A = absent.

		Chelicerae		Pedipalps		Body Line		Walking legs		Telescoping		
Author	Figure	E	R	E	R	C	St	Sp	F	P	A	Identification
typical carcass		-	+	-	+	-	+	-	+	-	+	carcass
typical molt		+	-	+	-	+	-	+	-	+	-	molt
Carvalho & Lourenco 2001	Figure 2	-	+	+	-	-	+	-	+	-	-	carcass
Lourenco & Gall 2004		-	-	+	-	-	+	-	+	-	-	molt
Santiago-Blay 1988	Figure 1–2	-	-	+	-	+	-	+	-	-	-	molt
Santiago-Blay et al. 2004	Figure 1	-	+	-	-	-	+	-	-	-	-	carcass

and Gaban & Farley (2002) noted that the walking legs become rigid and fixed in place before ecdysis begins. These observations suggest that ecdysial leg position may be retained after ecdysis, although Gaban & Farley (2002) also observed that *Paruroctonus mesaensis* (Stahnke 1957) pulls its appendages medially during ecdysis. The exuvia of all the scorpion genera examined in our study show the splayed walking leg posture.

The position of the pedipalps differs between the exuvia and carcasses examined for this study. Our observations compliment those of Gaban & Farley (2002), who noted that *Androctonus australis* (Linnaeus 1758) holds its pedipalps in a distinctive position before ecdysis, with the chelae sharply angled toward each other. These authors noted that this pre-molt posture contrasts with the posture of scorpions at rest, in which the pedipalps are retracted. More than half of the carcasses examined in this study retained their pedipalps in a retracted position, suggesting that the relaxed position also served as a post-mortem posture.

Observations on modern scorpions also support body line as a criterion for distinguishing exuvia (curved) from carcasses (straight). Gaban & Farley (2002) cite examples of exuvia of *A. australis*, *P. mesaenis*, and *Parabuthus transvaalicus* Purcell 1899 that are curved dorsally or to one side.

Fossil scorpion taphonomy.—We examined 24 well-preserved, nearly complete fossil scorpions from the Ciurca collection at the Yale Peabody Museum (YPM; Table 4) and applied our taphonomic criteria in an attempt to characterize each specimen as molt or carcass. One or more of the four distinguishing criteria (body line, position of chelicerae, position of walking legs, and position of pedipalps) could be identified in 85–90% of the YPM specimens (Table 4). Approximately 89% of the classifiable YPM specimens fit the criteria for molts (Table 4). The results of the YMP census support the conclusion of other authors that the bulk of the scorpion fossil record comprises exuvia (Kjellesvig-Waering 1986).

Most authors of papers on scorpion systematics do not include taphonomic data in their description of new specimens, nor do they make a determination of whether their illustrated specimens represent molts or carcasses. The taphonomic criteria developed herein can also be used to make the molt/carcass determination for fossil scorpions illustrated in the literature (Table 5).

The results of the tumbling experiments suggested that scorpion molts in water are nearly as durable as scorpion carcasses, as measured by time to total disarticulation (Tables 2, 3). This observation is interesting because it runs counter to the intuitive conclusion that molts must be more fragile than carcasses, and it calls into question the assumption that intact fossil scorpions are more likely the remains of the more "robust" carcass than the presumably "fragile" exuvium (Wills 1959; Jeram 2001; Gaban & Farley 2002).

The scorpion body plan is strikingly similar to that of the extinct eurypterids (Chelicerata: Eurypterida), and on this basis, scorpions can be considered reasonable taphonomic analogues for their extinct chelicerate cousins. Therefore, the criteria for distinguishing fossil scorpion molts and carcasses may also be useful for distinguishing fossil eurypterid molts and carcasses. A recent study of eurypterid molting (Tetlie et al. 2008) concluded that eurypterids indeed molted in much the same manner as modern scorpions, in spite of the obvious ecological differences between the aquatic eurypterids and the terrestrial scorpions.

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Temperature and desiccation effects on the antipredator behavior of *Centruroides vittatus* (Scorpiones: Buthidae)

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Abstract. Temperature can profoundly affect many physiological processes, including muscle performance. Many ectotherms appear sensitive to this relationship, choosing times and locations of activity permitting high body temperatures and, thus, quick escape from predators. High body temperatures, however, can lead to dehydration, which in turn affects muscle performance. Striped bark scorpions *Centruroides vittatus* Say 1821 provide an ideal model for assessing the effects of temperature and water loss on two potentially important antipredator behaviors, sprinting and stinging. Scorpions had significantly higher sprint speeds at warmer temperatures, with males significantly faster than females. Additionally, sting latency was longer and sting rate lower when scorpions were cooler. Intriguingly, females appear capable of stinging at a higher rate than males. Desiccation allowed the scorpions to sprint significantly faster than control (hydrated) scorpions, probably due to weight loss. The influence of temperature on sprinting and stinging might thus explain bark scorpions' preference for maintaining high body temperatures during periods when they are exposed to predation. When inactive, however, scorpions may benefit from maintaining lower body temperatures to decrease resting metabolic rate and desiccation.

Keywords: Defensive behavior, scorpions, thermal ecology, sting speed, sexual dimorphism

Ectotherms generally exhibit preferred body temperatures (T_p) that they maintain through behavioral thermoregulation. Temperature profoundly affects many physiological processes including, but not limited to, oxygen consumption, digestion (Bobka et al. 1981; Zhang & Ji 2004), growth (Angilletta et al. 2004), and locomotor performance (Forsman 1999). The latter process is related to temperature primarily due to well-recognized thermal dependencies of muscular contraction and relaxation (Bennett 1984). The influence of temperature on locomotor performance is ecologically important for many organisms, as it impacts both hunting ability and predator avoidance via such activities as sprinting (Waldschmidt & Tracy 1983; Bauwens et al. 1995), flying (Machin et al. 1962), swimming (Turner et al. 1985), and striking (Greenwald 1974; Rowe & Owings 1990; Webb & Shine 1998). Indeed, endothermic enemies are known to take advantage of ecotherms that find themselves with body temperatures (T_b) cooler than they might prefer (Rowe & Owings 1996; Swaisgood et al. 1999, 2003). Sprint speed, in particular, can be critical for an ectotherm attempting escape from a potential predator (van Berkum et al. 1986; Hertz et al. 1988). For this reason, T_p is often strongly correlated with the optimal temperature (T_o) for sprinting capacity (Miller 1982; Bauwens et al. 1995; Forsman 1999), providing evidence that ectothermic organisms typically select body temperatures that maximize their locomotor capabilities. If a prey organism's ability to survive encounters with and escape from predators is dependent on locomotor performance, then maintaining T_p will favor survival in the face of predation; indeed, this has been demonstrated in wild populations (Christian & Tracy 1981).

The match, however, between T_p and T_o is not always perfect (see reviews in Huey & Slatkin 1976; Huey 1982). Some ectothermic organisms may have a broad range of temperatures over which performance varies little (Schmalhofer & Casey 1999), negating the advantage of finely tuned behavioral thermoregulation. Other species may face competing physiological demands with different T_os. Side-blotched lizards (*Uta stansburiana*), for example, select microhabitats that maximize their sprint speeds during the morning and late afternoon, but chose sub-optimal, shaded habitats during midday to avoid desiccation (Waldschmidt & Tracy 1983). Water conservation can itself feed back on locomotor performance. Dehydration has been found to decrease endurance but not burst activity in frogs and lizards (Crowley 1985; Moore & Gatten 1989; Wilson & Havel 1989) and to decrease walking velocity in crayfish (Claussen et al. 2000). Because higher temperatures typically increase the rate of water loss (Slobodchikoff 1983), many ectotherms might face a difficult physiological trade-off: a high T_b may support increased performance through its effect on muscle contraction while simultaneously decreasing performance from desiccation.

Desert scorpions provide an excellent model organism for examining the effects of temperature and dehydration on antipredator motor patterns. They flourish within desert ecosystems (Hadley 1974), forming an ecologically important link as the dominant predators of small herbivorous and detritivorous arthropods (McCormick & Polis 1990) and as the prey of many vertebrates (Polis et al. 1981; Ayal 2007). Indeed, the total biomass of these highly successful arachnids can exceed that of all vertebrates combined in some desert systems (Polis 1990). Predation is the dominant mortality factor (Polis 1990), making scorpions especially valuable for studies of antipredator behavior. They tend to exhibit nocturnal, "time-minimizing" activity patterns (Schoener 1971) in which they are rarely active and foraging, preferring to remain in refugia and thus limiting their risk of falling prey (Polis 1980). Surface activity of many scorpions is highest during the earlier hours of the night and gradually decreases as dawn nears (Hadley & Williams 1968; Polis 1980; Warburg &

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Polis 1990; Carlson et al., unpublished data). This may afford them access to sun-warmed substrates, returning to refugia as the surface substrates cool. Additionally, scorpions appear most active during both warm (Polis 1980) and humid (Skutelsky 1996) weather conditions, decisions that may help them maximize body temperature while minimizing water loss.

Anecdotal reports suggest that temperature may indeed affect locomotor performance in scorpions (Hadley 1974; Warburg & Polis 1990), though there is, as yet, little experimental evidence of this. The water loss rates of scorpions, though low (Hadley 1974), are known to rise with temperature (Hadley 1970; Gefen & Ar 2006), and desiccated scorpions exhibit limited functionality of the limbs (Sensenig & Shultz 2004). This is likely due to the use of hydraulics in some locomotor activities (Sensenig & Shultz 2004) and the decreased effectiveness of hydrostatic systems with fluid loss (Anderson & Prestwich 1975). Additionally, dehydration may reduce the oxygen-transporting abilities of the hemolymph, further inhibiting locomotor performance (Gefen & Ar 2005). All of this serves to highlight the potential importance of thermoregulation and water balance for locomotor-based antipredator behaviors. Indeed, the primary defensive mechanisms of scorpions, sprinting (Shaffer & Formanowicz 1996, 2000) and stinging, fall into this category. The purpose of this research is to determine the effects of temperature and desiccation on these antipredator behaviors in a model scorpion.

Centruroides vittatus Say 1821, the striped bark scorpion, is an excellent species for such research. In addition to being the most common and active scorpion in many lithic deserts of the southwestern United States and northern Mexico (Brown et al. 2002), scorpions of the family Buthidae are noted for their high temperature preferences (Warburg & Ben-Horin 1981), low water loss rates (Gefen & Ar 2004), and well-developed osmoregulatory ability (Gefen & Ar 2005). *C. vittatus* is subjected to a wide range of temperatures, from searing summer heat to subfreezing winter nights (Whitmore et al. 1985). Striped bark scorpions appear sensitive to the influence of temperature on their defensive capabilities, as they are less likely to be active on the surface during cool weather (Brown & O'Connell 2000; Brown et al. 2002; Yamashita 2004). And when they are active, they are less likely to seek refuge in bushes (an antipredator tactic; Brown & O'Connell 2000; McReynolds 2004) when temperatures are warm (McReynolds 2008).

Striped bark scorpions are also appropriate because stinging and sprinting are important components of their antipredator behavior. Although bark scorpions (*Centruroides* spp.) are well known for possessing potent neurotoxins that might deter a vertebrate enemy, grasshopper mice (*Onychomys* spp.) are resistant to the lethal effects of the venom and feed voraciously on *Centruroides* (Rowe & Rowe 2006, 2008); an effectively delivered sting, however, often causes sufficient pain for the mouse to drop the scorpion, providing an opportunity for its escape (Rowe & Rowe 2006). The broad range of environmental temperatures to which striped bark scorpions are exposed, coupled with the importance of stinging and sprinting for evading predators, make *C. vittatus* an ideal model for testing hypotheses about scorpion thermal ecology and physiology in relation to defensive behavior. In another report (Carlson et al., unpublished data), we show that *C. vittatus* from two different populations both select substrates generating high body temperatures (36–38°C) when tested in a thermal gradient.

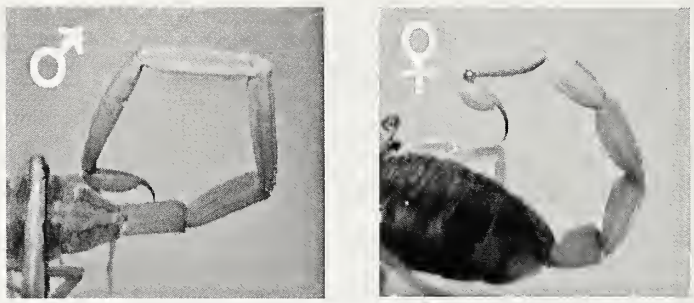

Figure 1.—Sexual dimorphism in the metasomas of male and female *C. vittatus*.

Here, we examine the effects of temperature, and of temperature-induced dehydration, on their defensive ability.

METHODS

Animals.—From a large group (600+) of *Centruroides vittatus* scorpions collected during May 2008 in the Organ Mountains in New Mexico, USA, 180 experimental subjects (60 adult males, 60 adult females, and 60 unsexed juveniles) were selected randomly. All scorpions were kept at room temperature (~25° C) in plastic sweater boxes with gravel substrates, egg crate refugia, and petri dishes for water. The communal nature of *C. vittatus* (McAlister 1966; Polis & Lourenço 1986) allowed us to house 30–60 individuals per box with no signs of aggression. Due to concerns that the added weight and the energetics of digestion may influence thermal ecology, the scorpions were only fed between experiments (i.e., after all trials for a single experiment were completed), and all were fed at the same time. Food items offered included crickets (*Acheta domesticus*), "superworms" (*Zoophobas morio* larvae), and mealworms (*Tenebrio molitor* larvae). To best approximate the light conditions faced by scorpions in the wild (where they are active at night and hiding in crevices or burrows during the day), lights in the room housing the scorpions were kept on 24-h D; a small window nonetheless provided diffuse daylight cueing.

The scorpions were each marked with a unique four-dot pattern of fluorescent paint (Hadley & Williams 1968; Bradley 1988). Individuals were sexed by the length and thickness of the segments of the metasoma (tail). Males have thin and elongate segments whereas females possess shorter and thicker segments (Polis & Sissom 1990; Fig. 1). Pregnancies in females to be tested were noted; because most of the females were gravid (> 90%), this variable could not be avoided while maintaining adequate sample sizes. Individuals were characterized as juveniles based on qualitative attributes, such as a lack of clear sexual dimorphism and smaller total body sizes and masses.

Temperature effects on sprint speed.—All 180 scorpions were used in the sprint speed trials. Because there is significant and heritable variation in sprint speed ability between individuals (Shaffer & Formanowicz 2000), each scorpion was tested at every temperature and compared with its own performance (repeated measures). The scorpions were divided equally into three subgroups (each containing 20 adult males, 20 adult females, and 20 unsexed juveniles) to systematically counterbalance treatment effects (i.e., temperatures) across trials. Trials on the same individual scorpion were separated by a minimum of 48 h.

The sprint speed of each scorpion was assessed at three temperatures; T_p = 38° C (Carlson et al., unpublished data), 25° C, and 10° C. Test temperatures were chosen to span the range of temperatures experienced by free-ranging striped bark scorpions (Brown et al. 2002; Yamashita 2004; Carlson et al., unpublished data).

Sprint speed trials were performed using the same gradient track unit employed in earlier temperature preference trials (Carlson et al., unpublished data), consisting of a 3.2-mm thick copper base, 1.22 m long by 30.5 cm wide. 10.2-cm-tall aluminum sides enclosed the base and also divided the unit lengthwise into three separate tracks, each approximately 10.2 cm wide by 1.22 m long. The base was covered with approximately 1 cm of fine sand. The experimental temperatures were achieved by placing the track, with its copper bottom, on six hot plates running the length of the unit for the 38° C trials, and on a continuous line of ice packs for the 10° C condition; since the experimental room was kept as close as possible to 25° C, no heating or cooling was needed for this latter treatment. All of the scorpions to be tested at a given temperature were individually contained in small acetate cylinders (~ 6 cm × 6 cm, constructed from overhead transparency sheets) placed in one of the tracks to prevent them from becoming fatigued from excessive movement. They were acclimated to the temperature of the unit for at least 6 min; pilot tests, performed with a Sensortek BAT-12 thermocouple connected to a Harvard Apparatus microprobe inserted into the mesosoma (abdomen), show this is more than sufficient time for a scorpion's T_b to reach the temperature of the substrate upon which it rests. The temperature of the substrate (T_s) inside each scorpion's acetate containment cylinder, and thus the approximate T_b of that scorpion, was taken remotely (preventing disturbance to the scorpion) using an Extech model RH101 infrared thermometer immediately prior to sprint testing.

Sprint speed trials were conducted in the two tracks not used for containment/acclimation. Because of negative phototokinetic behaviors in scorpions (Abushama 1964; Warburg & Polis 1990), the end of the track was shaded while the beginning was well-lit to encourage unidirectional movement. Scorpions were placed on the track and, if necessary, startled with a tap on the metasoma to induce running. A stopwatch accurate to 0.01 s was used to record the time elapsed from crossing the starting line to either stopping or reaching a 50 cm "finish line" marked on the track. The distance at the final point was measured for individuals who stopped short. Each individual was induced to sprint three times at each of its test temperatures; within a trial (i.e., test temperature), scorpions were allowed to rest for several minutes between successive sprints. The highest speed achieved by an individual in its three sprints at a given test temperature was used for later analysis. Three individuals (two juveniles and one adult male) that refused to sprint during one or more of its test temperatures were removed from the analysis, reducing the sample size to 177. The sand in the apparatus runway was shifted and smoothed to even out the surface between tests on different individuals.

Temperature effects on sting speed.—A subset of 54 individuals (18 adult females, 18 adult males, and 18 unsexed juveniles) of the original 180 marked scorpions from the sprint speed trials were selected for sting trials. In the same manner as the sprint speed measurements, these scorpions were split into three equal-sized subgroups that were tested at all three temperatures in counterbalanced order. Once again, successive trials on the same scorpion were separated by a minimum of 48 h. One adult male died before completing all three of his trials and was removed from the analyses.

Trials were conducted using a 9.5-mm thick copper plate, approximately 19 cm × 16 cm, on top of which was placed a thin layer of fine sand. An acetate cylinder (approximately 7 cm tall, 8 cm long and 2.5 cm wide at the center) similar to those used in the sprint trials was used to contain the scorpion, with the simple modification of partially flattening the cylinder into an ellipse to keep the scorpion oriented perpendicularly to the video camera (see below). Test temperatures were induced by placing the copper plate on ice (10° C), on the lab bench (25° C), or on a hot plate (38° C), using insulation and spacers to fine-tune the temperature. A sting-eliciting probe was constructed from a 15-cm stick, onto which a 2-cm × 2.5-cm piece of index card was affixed to one end as a target.

Scorpions were placed individually into the acetate ellipse and allowed 6 min to equilibrate to the temperature of the testing apparatus. As with sprint trials, the T_s inside each scorpion's acetate ellipse was taken immediately preceding its sting test. Each test was filmed with a Canon XL2 digital video camera at 30 fps. To elicit a sting, the probe, target end down, was lowered slowly from above and pressed firmly on the 4th or 5th tergite (dorsal cuticular segment) of the scorpion; both the scorpion and the target were oriented perpendicular to the camera. The probe was held in place for at least two seconds after the scorpion stung. Scorpions that did not sting within 10 s were characterized as failing to sting and the trial was terminated.

Video was analyzed frame-by-frame. Elapsed time from first contact of the probe with the scorpion's body to the moment when the scorpion's aculeus (stinger) touched the target was recorded as sting latency. The number of separate contacts between the aculeus and the target (i.e., number of stings) in the following 2 s was used to calculate a sting rate (stings/s). Individuals whose aculeus failed to contact the target were excluded from sting latency analyses. Occasionally, a scorpion's aculeus stuck in the target, or lodged between the target and the scorpion's body; individuals whose aculeus remained stuck for more than 1 s of the 2-s "post-first-sting" filming window were excluded from sting rate analyses.

The speed with which a scorpion can deliver a sting might be influenced not only by temperature, age, or gender, but also by the starting position of its telson (the last tail segment, to which the aculeus is attached) and its metasoma (tail). We therefore used the videotapes to quantify the starting position of each scorpion's telson along its longitudinal axis, employing an ordinal scale from 1–7 (Fig. 2a), generating an estimate of metasomal "curling"; similarly, we used a three-level ordinal scale to quantify the lateral (sagital) angle of each scorpion's metasoma (Fig. 2b) just prior to being touched with the sting stimulus.

Dehydration effects on sprint speed.—From the marked scorpions that were not employed in the sting experiments, 60 individuals were randomly selected and divided into a control and a treatment group, each with 10 adult males, 10 adult females, and 10 juveniles. All scorpions were measured again

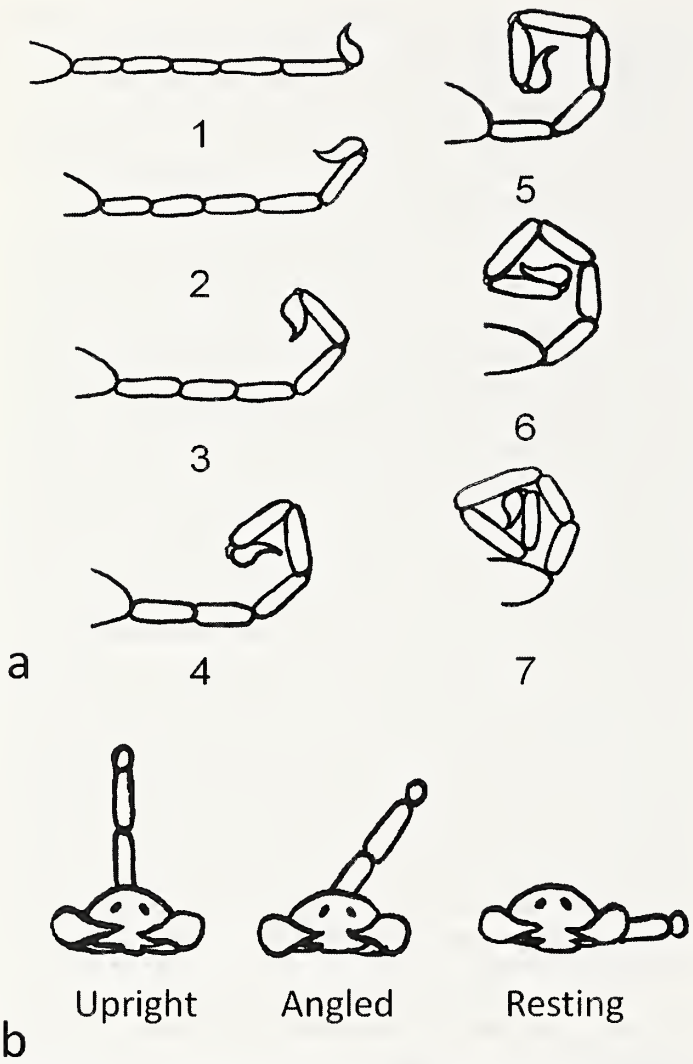

Figure 2.—Ordinal system for quantifying a) degrees of metasomal curling and b) metasomal angle in *C. vittatus* just prior to delivering a sting.

for sprint speed at room temperature (25° C) using the same procedure described earlier. The treatment group was then placed in a glass desiccator with 3.2 cm of Drierite layering the bottom; a porcelain plate covered with a paper towel was used to prevent scorpions from making direct contact with the desiccant. The entire unit, desiccator plus scorpions, was then placed inside a Percival model I36LLC8 environmental chamber for 70 h at 36° C. The control group was placed in a twin desiccator, again with a plate and paper towel barrier, but with petri dishes of water supplied for drinking and no Drierite. The control group was kept in the environmental chamber during the same period as the experimental group, for which mass losses of 10–20% were targeted.

After desiccation, all the scorpions were again tested at 25° C for sprint speed. The change in a scorpion's sprint speed was calculated as a percent of its original speed. The paper towel and porcelain plate barrier was not entirely effective; scorpions that slipped past the barrier and came in contact with either the desiccant or water condensed on the bottom were excluded from subsequent analyses, reducing the control and treatment group sample sizes from 30 each to 19 and 22, respectively.

Statistical analysis.—A mixed-design, repeated measures ANOVA, with one between-group effect (age/sex) and one within-subject effect (temperature), was performed on the sprint speed, sting speed, and sting rate data. The possible influences of sensitization, habituation, or fatigue across trials was assessed by substituting "trial number" for "temperature" in a second set of ANOVAs. Dependent variables (DVs) in each of these six ANOVAs were tested for the standard parametric assumptions of normality, homogeneity of variances, and repeated-measures sphericity (Keppel 1991). Two of the six DVs (sprint speed and sting latency with temperature) violated the sphericity assumption and were thus subjected to the conservative Greenhouse-Geisser correction (Keppel 1991, Field 2005). One DV (sting latency with temperature) violated the normality and homogeneity assumptions and could not be corrected using a data transformation; we therefore applied a conservative level of significance ($P < 0.01$) to this DV to minimize any risk of Type I error (Keppel 1991). Effect sizes (strengths of association) are reported as partial η^2 (partial eta squared). Multiple comparisons for any of the mixed-design ANOVAs with significant main effects were conducted using Bonferroni's adjustments, the preferred post-hoc test for repeated measures (Field 2005).

Two-way independent ANOVAs were used to assess the influences of dehydration and age/sex on two dependent variables, the change in a scorpion's body mass and the change in its sprint speed following ~ 3 days at $T_b = 36°$ C. Both DVs were tested for the standard parametric assumptions of normality and variance homogeneity. Sprint speeds were significantly non-normal and could not be transformed to meet this assumption; we therefore applied a conservative level of significance ($P < 0.01$) to this DV to minimize the risk of Type I error (Keppel 1991). Effect sizes are reported as partial η^2; Bonferroni adjustments were again used to assess the significance of multiple comparisons.

Two dichotomous variables were analyzed using the Cochran's Q test for repeated measures (Siegel 1956): whether or not the scorpion sprinted the full 50 cm or stopped short; and whether or not a scorpion's sting "hit" the target. The influence of the starting positions of a scorpion's telson (metasomal curling) and metasoma (metasomal angle) on sting latency were analyzed with a Friedman's non-parametric repeated measures ANOVA, followed by post-hoc tests using Wilcoxon's signed-rank tests (Siegel 1956; Field 2005). The effect of metasomal curling and metasomal angle on sting latency was further explored by conducting, for each test temperature separately, a non-parametric Spearman's rank order correlation (Siegel 1956; Field 2005).

The computer program SPSS (Version 15.0, Chicago, Illinois) was used to perform statistical analyses. Descriptive results are presented in the text as means +/− 1 SE.

RESULTS

Sprint distances.—The experimental protocol was very effective in eliciting "straight-line" sprints from all 177 scorpions tested at each of their three body temperatures. Indeed, 88% of the trials (467/531) resulted in the scorpion sprinting the full 50 cm marked on the track. The average distance of the 64 sprints (12%) that stopped short of the 50-cm mark did not differ considerably across the three test temperatures (30.4 ± 9.14 cm at 10° C, 35.1 ± 5.37 cm at 25°

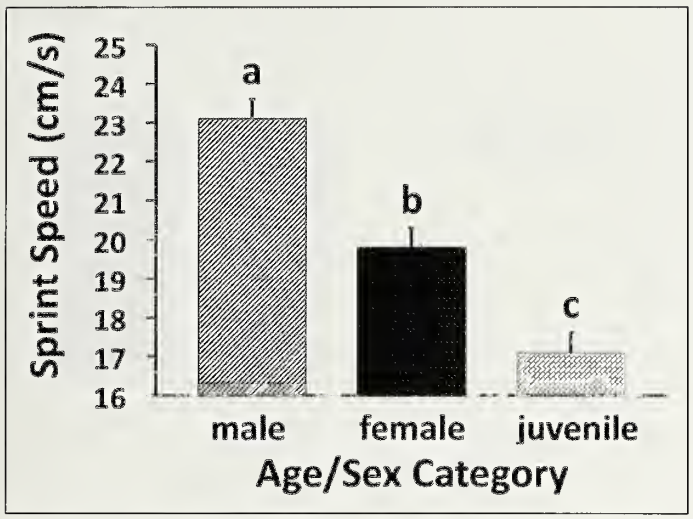

Figure 3.—Mean + SE sprint speeds of *C. vittatus* as a function of age and gender. Different letters signify different mean values at $P < 0.05$.

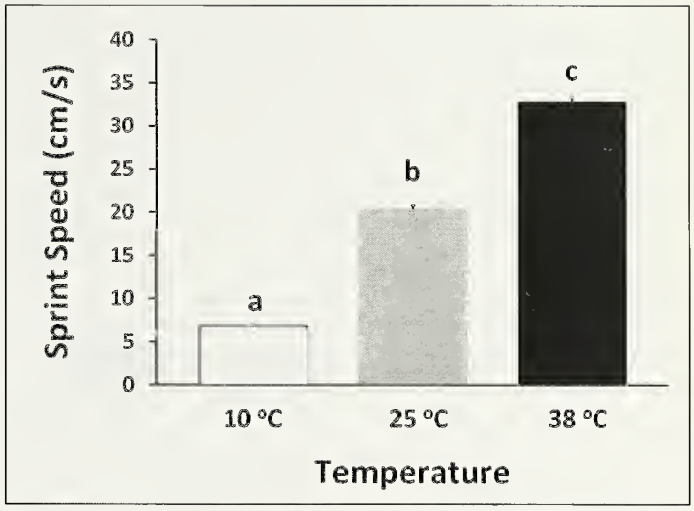

Figure 4.—Mean + SE sprint speeds of *C. vittatus* as a function of temperature. Different letters signify different mean values at $P < 0.05$.

C, and 35.0 ± 6.20 cm at 38° C, respectively). The proportion of "short sprints" was, however, significantly influenced by a scorpion's body temperature: only 10/177 tests (5.65%) at 25° C and 5/177 tests (2.82%) at 38° C resulted in the scorpion stopping short, compared to 49/177 (27.68%) truncated runs for scorpions at 10° C ($Q_2 = 64.48$, $P < 0.001$). Moreover, the 10 shortest runs (ranging from 10–23 cm) recorded in all 531 trials were for scorpions tested at their coolest temperature.

Sprint speeds.—There was a highly significant difference in the sprint speeds between each age/sex category ($F = 34.2$; $df = 2$, 174; $P < 0.001$; Fig. 3), with males sprinting fastest (23.1 ± 0.51 cm/s), followed by females (19.8 ± 0.50 cm/s) and then juveniles (17.1 ± 0.51 cm/s); the partial η^2 for this main effect was 0.282. Post-hoc Bonferroni comparisons show that male sprint speeds were significantly faster than either females or juveniles ($P < 0.001$), while females were faster than juveniles ($P = 0.001$). It is worth noting that 57 of the 60 female scorpions involved in these sprint tests were gravid; when we removed the three non-gravid females (one each from the three subgroups of adult females) from the analysis, results were unchanged.

Temperature significantly affected sprint speeds ($F = 1712.42$; $df = 1.45$, 252.67; $P < 0.001$; Fig. 4): at the warm temperature (38° C), scorpions averaged speeds of 32.8 ± 0.65 cm/s; at medium temperatures (25° C), their speed averaged 20.4 ± 0.37 cm/s; at the cooler temperature (10° C), speeds averaged only 6.8 ± 0.15 cm/s. The partial η^2 for this main effect was 0.908. Post-hoc Bonferroni comparisons revealed significant differences between each of the temperature treatments ($P < 0.001$). The positive effects of increasing body temperature on sprint speeds appears more pronounced in males than in females and juveniles, as evidenced by a small (partial $\eta^2 = 0.142$) but nonetheless significant interaction between age/sex and temperature ($F = 14.36$; $df = 2.94$, 252.67; $P < 0.001$). There was no significant effect of trial number on sprint speed ($F = 0.42$; $df = 2$, 348; $P = 0.657$), nor was there a significant interaction between trial number and age/sex ($F = 0.93$; $df = 4$, 348; $P = 0.983$).

Although our test apparatus was unsophisticated (i.e., a copper-bottomed runway placed on ice bags, a counter top, or hot plates), the variability in our targeted treatment temperatures (10, 25, and 38° C) was small; the T_s (and, thus, the T_b) for scorpions during their 10° C trial was 10.4 ± 0.20° C; for scorpions during their 25° C trial it was 25.4 ± 0.05° C; and for scorpions in their 38° C trial, it was 40.9 ± 0.08° C. The low variability in substrate temperatures, recorded as they were along the full length of the runway, attests to the effectiveness of the copper plate in diffusing temperatures evenly across the apparatus.

Sting speeds and effectiveness.—Eliciting stings proved more difficult than inducing sprints. Indeed, 24.5% of the 159 sting trials ended with the scorpion either not stinging or attempting to sting but missing the target. Temperature appeared to influence stinging success, as 84.9% and 75.5% of the stings delivered by scorpions when at 25° C and 38° C T_b hit the target, respectively, while only 66.0% of the stings delivered by scorpions at 10° C were so effective ($Q_2 = 6.25$, $P = 0.044$).

For those scorpions who managed to sting the target in each test, temperature had a significant effect on their latency to sting ($F = 13.27$; $df = 1.06$, 23.26; $P = 0.001$; Fig. 5); cooler scorpions (10° C) took significantly longer (Bonferroni adjustment; $P < 0.001$) to deliver a sting (1.67 ± 0.40 s) than either the medium (25° C; 0.29 ± 0.05 s) or warm (38° C; 0.32 ± 0.08 s) scorpions, which did not themselves differ. The partial η^2 for this main effect was 0.376. The age and sex of the scorpion had no effect on sting latency ($F = 1.44$; $df = 2$, 22; $P = 0.258$), nor was there a significant interaction between age/sex and temperature ($F = 1.77$; $df = 2.11$, 23.26; $P = 0.191$). Note that 17 of the 18 female scorpions included in this analysis were gravid; when the non-gravid female was removed from the analysis, results were unchanged.

The significant influence of T_b on sting latency is unlikely to be the result of systematic differences in the initial positions of a scorpion's telson or metasoma. There were no significant differences in metasomal curling ($\chi^2 = 2.14$; $df = 2$; $P = 0.343$) or angle ($\chi^2 = 3.60$; $df = 2$; $P = 0.166$) across the three test temperatures. Moreover, neither metasomal curling ($r_s = 0.218$; $P = 0.208$) nor angle ($r_s = 0.053$; $P = 0.763$) was significantly correlated with sting latency for scorpions during their 10° C test; results at 38° C were similar, with insignificant

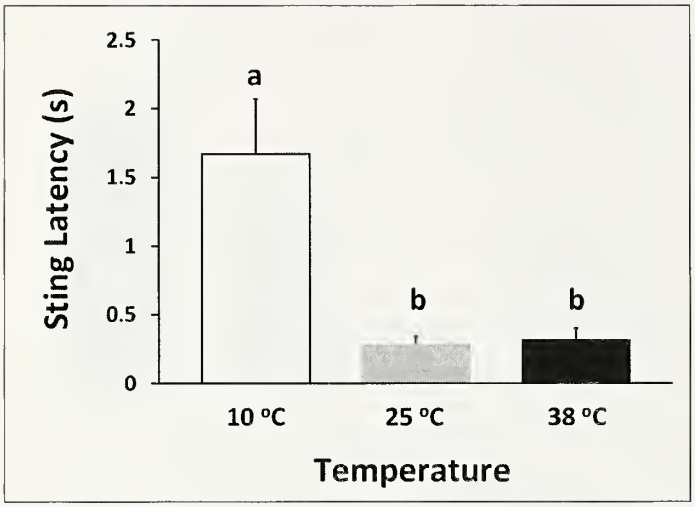

Figure 5.—Mean + SE sting latencies of *C. vittatus* as a function of temperature. Different letters signify different mean values at $P < 0.05$.

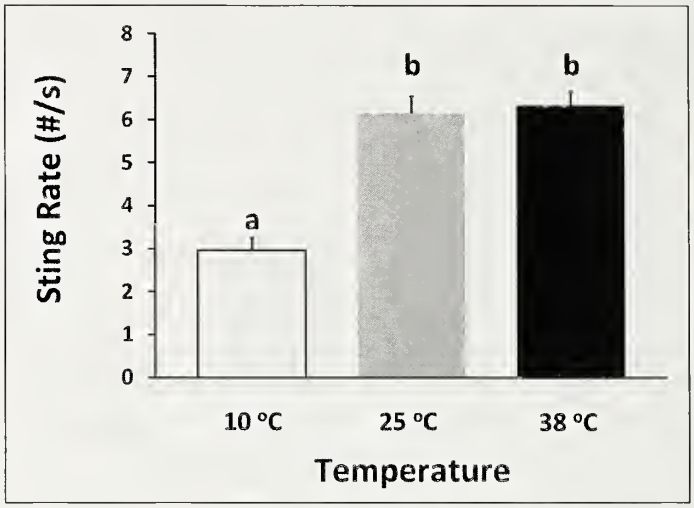

Figure 6.—Mean + SE sting rates of *C. vittatus* as a function of temperature. Different letters signify different mean values at $P < 0.05$.

correlations of sting latency with metasomal curling (r_s = 0.049; P = 0.762) and angle (r_s = 0.077; P = 0.637). Intriguingly, both curling and angle were significantly correlated with sting latency for scorpions at intermediate body temperatures; e.g., at 25° C, scorpions with higher values of metasomal curling (positions 4–6 in Fig. 2a; note that no scorpion had a curling value of 7 at this temperature) delivered slower stings (r_s = 0.449; P = 0.002) than individuals with lower values (positions 1–3 in Fig. 2a). Similarly, individuals whose metasomas where held at ~ 45° delivered slower stings than those holding them vertically (r_s = 0.491; P = 0.001); only one individual at 25° C held its metasoma in a resting position (Fig. 2b), preventing any meaningful comparison for this angle. There was no significant effect of trial number on sting latency (F = 0.34; df = 2, 44; P = 0.717), nor was there a significant interaction between trial number and age/sex (F = 1.26; df = 4, 44; P = 0.301).

A similar pattern was found in the effects of temperature on sting rate (F = 41.43; df = 2, 86; $P < 0.001$; Fig. 6); cooler scorpions (10° C) delivered significantly fewer (Bonferroni adjustment; $P < 0.001$) stings per second (2.97 ± 0.27) than did scorpions at intermediate (25° C; 6.16 ± 0.39 stings/s) and warm (38° C; 6.33 ± 0.32 stings/s) body temperatures, which themselves did not differ. The partial η^2 for this main effect was 0.491. And while there was no significant interaction between age/sex and temperature on sting rate (F = 0.32; df = 4, 86; P = 0.864), the main effect of age/sex approached significance (F = 2.81; df = 2, 43; P = 0.072). Though caution is merited, it is noteworthy that female bark scorpions appear to deliver rapid, probing stings (5.90 ± 0.39 stings/s) more quickly than either juveniles (4.86 ± 0.38 stings/s) or males (4.71 ± 0.39 stings/s). Removing the sole non-gravid female from sting rate analyses did not influence these results. There was no significant effect of trial number on sting rate (F = 0.58; df = 2, 86; P = 0.561), nor was there a significant interaction between trial number and age/sex (F = 0.44; df = 4, 86; P = 0.779).

Our test apparatus for assessing sting speed and sting rates was, like our apparatus for assessing sprint speeds, rather crude. It was, nonetheless, effective and reliable at generating our targeted test temperatures. The T_s, and thus T_b, for scorpions tested at their targeted T_b of 10° C was 9.4 ± 0.18° C; for scorpions during their 25° C trials, T_s was 25.0 ± 0.05° C; and for 38° C trials, T_s was 37.8 ± 0.09° C.

Dehydration effects.—The scorpions receiving the desiccation treatment lost a significantly greater percentage of their body mass (−14.95 ± 1.3%) than did control scorpions (−0.44 ± 0.9%; F = 78.32; df = 1, 35; $P < 0.001$; Fig. 7). The partial η^2 for this main effect was 0.691. The effect of age/sex on mass loss was insignificant (F = 0.97; df = 2, 35; P = 0.391), as was the interaction of dehydration/control with age/sex (F = 0.41; df = 2, 35; P = 0.670).

The effect of hydration on sprint speeds was highly significant (F = 22.84; df = 1, 35; $P < 0.001$; Fig. 8); desiccated scorpions increased their sprint speed by +7.57 ± 7.1% between their pre- and post-tests, while control scorpions decreased in speed by −27.69 ± 4.8%. The partial η^2 for this main effect was 0.395. At first glance, the age and sex of a scorpion also appear to influence how its sprint speed changed (F = 3.68; df = 2, 35; P = 0.035); females suffered the greatest

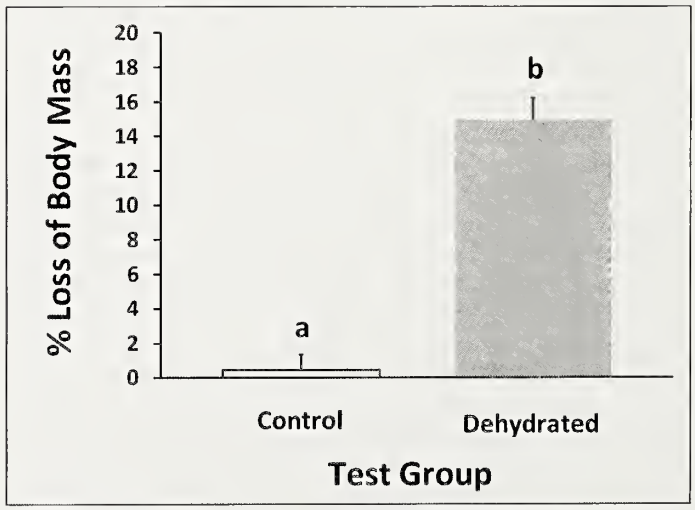

Figure 7.—Mean + SE percent of body mass lost in dehydrated and control *C. vittatus*. Different letters signify different mean values at $P < 0.05$.

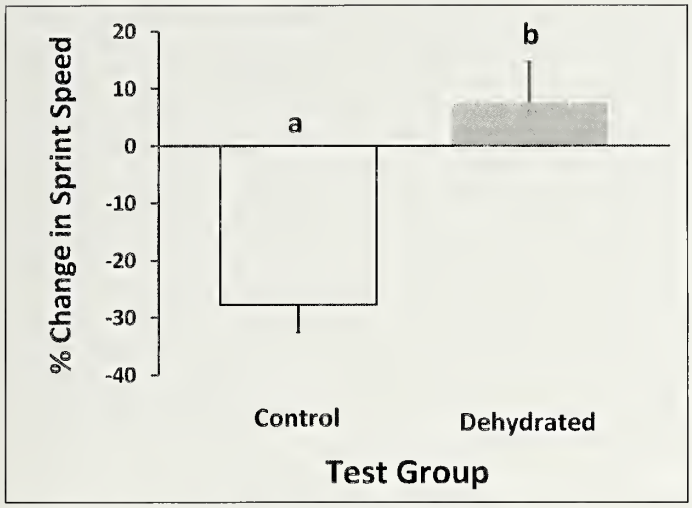

Figure 8.—Mean + SE percent changes between pre- and post-treatment sprint speeds for dehydrated and control *C. vittatus*. Different letters signify different mean values at $P < 0.05$.

reduction in sprint speeds (−19. 52 ± 6.5%) during their time in the environmental ehamber, followed by males (−9.80 ± 6.8%), while juveniles actually ran faster (+10.17 ± 8.8%) after spending ~ 3 days at 36° C. Note, however, that this DV did not meet the parametric assumption of normality, forcing us to reduce the level of significance (to $P < 0.01$) to minimize Type I error; by this more stringent criterion, age/sex had no significant influence on sprint speeds following desiccation. The highly significant effect of hydration, but not age/sex, on changes in scorpion sprint speeds was confirmed by running a Kruskal-Wallis non-parametrie ANOVA (Siegel 1956; Field 2005) on both variables; the former DV remained highly significant ($\chi^2 = 14.37$; $df = 1$; $P < 0.001$), while the latter was not ($\chi^2 = 2.33$; $df = 2$; $P = 0.312$). And finally, the interaction between age/sex and dehydration/control was insignificant ($F = 1.21$; $df = 2, 35$; $P = 0.31$).

DISCUSSION

Carlson et al. (unpublished data) show that striped bark scorpions from two widely separated populations inhabiting ecologically distinct habitats (one a rocky scrub desert in the foothills of the Organ Mountains in southern New Mexico, the other from the piney woods region of southeastern Texas) exhibit activity patterns and microhabitat preferences favoring high T_b's; moreover, when tested in a thermal gradient in the laboratory, scorpions from both populations had very high T_p's (36–38° C). The results presented here may help explain why; i.e., a *C. vittatus* that is warm sprints significantly faster and frequently sprints farther, delivers a quicker and more accurate sting, and delivers more stings per second than when it is cold. The influence of body temperature on this suite of defensive behaviors is the most robust finding from this study, as demonstrated by the uniformly higher effect sizes (partial η^2) for temperature than for age and gender. Temperature appears to have a more profound effect on sprint speed than sting efficacy, as evidenced by 1) the larger effect size (partial $\eta^2 = 0.908$) for maximal sprint velocity than for sting latency (0.376) or sting rate (0.491); and 2) by the significant differences (Bonferroni multiple comparisons) in sprint speeds across all three body temperatures (10, 25, and 38° C), but only between the coldest (10° C) vs. two warmer (25 and 38° C) temperatures for sting latency and rate. The preference of striped bark scorpions for the hottest of these temperatures may nonetheless make sense; when in the clutches of a grasshopper mouse, even a moderately warm scorpion can deliver a painful sting that will get it dropped, but only the warmest and fastest scorpion might then sprint to safety before the mouse re-attacks (Rowe & Rowe 2006).

Desiccation also had a significant effect on sprint velocity, but not in the manner we originally predicted. Surprisingly, scorpions that had lost nearly 15% of their body mass due to dehydration ran faster than they did prior to desiccation; control scorpions, whose body masses barely changed over the same three-day period while housed at 36° C, had sprint speeds ~ 1/3rd lower than their initial runs. The lack of any substantive effects of desiccation on sprinting may have several explanations. First, scorpions as a group use hydraulic pressure less in limb extension than most other arachnids (Shultz 1992), suggesting that fluid loss might have little impact on running speed. At high (though unspecified) levels of desiccation, however, scorpions do have difficulty moving (Sensenig & Shultz 2004); it is therefore possible that the amount of water loss in our trials was too low to have produced noticeable locomotor effects. Second, scorpions may, when suffering from dehydration stress, shift to anaerobic metabolism (Gefen 2008), which is less sensitive to the debilitating effects of desiccation than is aerobic metabolism (Weinstein 1998), allowing scorpions to maintain comparable levels of performance across varying levels of hydration. Finally, desiccation appears to affect activity bursts, such as sprinting, much less than it does endurance (Crowley 1985; Wilson & Havel 1989) mediated again through the different sensitivities of anaerobiosis vs. aerobiosis to desiccation. This study was not designed to measure endurance, so conclusions eannot presently be drawn about any possible inhibitory effects of desiccation on scorpion locomotor performance over longer time frames. The small positive effect of dehydration on scorpion sprint speeds (+ 7.6% faster following desiccation) is most likely the result of their significant loss of body mass (Crowley 1985).

In contrast, the dramatic reduction in sprint speeds for the control scorpions may reflect the negative physiological effects of supporting a high T_b (36° C) for a moderately long duration (70 h) without feeding. Studies have shown that ectotherms subject to high temperatures, but within the normal range they experience in the field, suffer denaturation of cellular proteins (Hofmann & Somero 1995); replacing these proteins requires energy that must be diverted from other tasks including, perhaps, maintaining the structures required for limb extension and retraction. Moreover scorpions, like many ectotherms, exhibit temperature-sensitive metabolic rates (Lighton et al. 2001); the energetic demands of higher T_bs would reduce glycogen stores (Sinha & Kanungo 1967), further squeezing the pipeline that fuels locomotion. Whether desiccated scorpions are buffered from protein damage at high T_bs is unknown. Dehydration, however, might actually protect scorpions from depleting their carbohydrate reserves, as desiccation significantly reduces their metabolic rates (Gefen 2008). Thus, for striped bark scorpions, the cost of maintain-

ing a high T_b is imposed not by desiccation, to which they appear well adapted, but through exhaustion of their energy resources. This cost may help explain the lengthy hiatus that scorpions spend between meals (Bradley 1982; Quinlan et al. 1993) in presumably cool refugia. Although speculative, proximate cues inducing a bark scorpion to leave the safety of its shelter could be a loss of body mass and/or a level of desiccation that maximize its sprint speed.

The influence of age and gender on the defensive behavior of striped bark scorpions also produced both anticipated and novel results. In a simple but elegant study, Shaffer and Formanowicz (1996) demonstrated that non-gravid female *C. vittatus* had significantly faster sprint speeds than gravid females, who themselves were faster than females carrying first-instar larvae on their backs. Moreover, female sprint speed was inversely related to the weight of the clutch they were carrying, either internally or dorsally. Given that all but three of the 60 females used in our sprint speed trials were gravid (and thus heavy, at 0.64 ± 0.016 gm), it is not surprising that their sprint speeds were significantly slower than the much leaner males (0.40 ± 0.12 gm). Juveniles, although light (0.17 ± 0.007 gm), were significantly slower than either males or females, likely resulting from their shorter limbs or incomplete motor development. Our results demonstrating both age and gender effects on sprint speeds thus support and extend the original findings of Shaffer and Formanowicz (1996). Uniquely, however, our results may help explain the dramatic sexual differences in *Centruroides* metasomas (Fig. 1) by pointing, tentatively, to a sexual dimorphism in antipredator behavior. Gestation in *C. vittatus* lasts eight months (Polis & Sissom 1990), a lengthy and risky period for female bark scorpions. Limited by the weight of their larger bodies and developing embryos, and thus incapable of achieving the sprint speeds of males, female *C. vittatus* may have selectively compensated for their increased vulnerability with better stinging ability. The longer, thinner metasomas of males appear to limit their ability to deliver fast, repetitive stings. While possession of a tail morphology capable of probing an enemy's epidermis for a weak spot certainly makes sense for a sluggish female bark scorpion, its absence in males is perplexing. Perhaps the longer and thinner metasoma of males enhances maneuverability, complementing their reliance on quick sprints to safety. Thin tails with an extended reach might also prove useful in courtship, or in male-male combat. We hope to explore these and other functional explanations concerning sexual differences in bark scorpion morphology and defensive behavior in future projects.

Our results documenting the influence of temperature on the defensive behavior of bark scorpions appear relevant to two additional, related aspects of their ecology; namely, their "errancy" and their proclivity for climbing bushes. Bark scorpions (*Centruroides* spp.) have earned the ecomorphological label of "errant" (McCormick & Polis 1990; Polis 1990) because, when hunting, they actively search for prey, quite unlike the ambushing strategy adopted by the majority of scorpion species. A cursorial foraging mode would, we argue, be more likely to attract the attention of visually hunting predators (e.g., grasshopper mice, *Onychomys* spp.; see Rowe & Rowe 2006) than would lying in wait. The foraging behavior of bark scorpions appears sensitive to their greater risk of predation, as evinced by their tendency of climbing into bushes to evade their enemies (Brown & O'Connell 2000), a behavior that becomes even more pronounced when the moon is bright (McReynolds 2004). Intriguingly, one report suggests that female bark scorpions are more likely than males to use bushes (Yamashita 2004), perhaps reflecting the females' greater vulnerability imposed by their slower sprint speeds. In a recent report, McReynolds (2008) shows that striped bark scorpions shift from foraging in vegetation to foraging on the ground when nighttime air temperatures are extremely warm (> 30° C); the author suggests this shift may be due to increased availability of terrestrial prey during the hottest months of the year. A complementary interpretation is that bark scorpions are more likely to be found foraging on the ground when temperatures are warm enough to permit quick sprints to safety.

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Cytogenetics of three species of scorpions of the genus *Brachistosternus* from Argentina (Scorpiones: Bothriuridae)

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Abstract. Meiotic studies on three phylogenetically distant species of the genus *Brachistosternus* Pocock from Argentina were conducted. One species is from the subgenus *Ministernus* Francke 1985, *B. ferrugineus* Thorell 1876, and two species are from the subgenus *Brachistosternus* Pocock 1893, *B. montanus* Roig-Alsina 1977 (Andean species group), and morphologically different populations of *B. pentheri* Mello-Leitão 1931 (plains species group). All species showed achiasmatic meiosis, absence of heteromorphic bivalents, and bibrachial and monobrachial chromosomes of different sizes. Males of *Brachistosternus ferrugineus*, *B. montanus,* and one population of *B. pentheri* have $2n = 46$. Males of the typical populations of *B. pentheri* have $2n = 42$. These results suggest that *B. pentheri* may comprise two species.

Keywords: Achiasmatic meiosis, Neotropics

The family Bothriuridae contains about 150 described species; it shows a Gondwanan distribution and has diversified mainly in southern South America. The systematics of this family has been resolved satisfactorily and the taxonomic status of most genera and species is well defined (Prendini 2003; Ochoa 2004a; Ojanguren-Affilastro & Ramírez 2009), making it particularly suitable for studying patterns of cytogenetic evolution. Chromosome number in Bothriuridae varies between 28 and 50 (Piza de Toledo 1947; Ferreira 1968; Giacomozzi 1977). Cytogenetic analyses have been performed on seven species, four species from the genus *Bothriurus* Peters 1861, *Timogenes elegans* (Mello-Leitão 1931) and two species of *Brachistosternus* Pocock 1893 (*B. pentheri* Mello-Leitão 1931 and *B. alienus* Lönnberg 1898) (Piza de Toledo 1947; Ferreira 1968; Giacomozzi 1977). The present study focuses on exploring cytological diversity among species and populations of *Brachistosternus* from Argentina.

Brachistosternus is the most diverse genus of the family, with about 40 known species (Ochoa 2002, 2004b; Ochoa & Acosta 2002; Ojanguren-Affilastro 2003a, b, 2005a, b; Ochoa & Ojanguren-Affilastro 2007; Ojanguren-Affilastro et al. 2007a, b; Ojanguren-Affilastro & Scioscia 2007). It inhabits arid areas in the western and southern parts of South America, from Ecuador (Cekalovic 1969) to southern Argentinean Patagonia (Ojanguren-Affilastro 2003b). The genus is divided into two subgenera (Ojanguren-Affilastro & Ramírez 2009), namely *Brachistosternus* Pocock 1893 and *Ministernus* Francke 1985. Both subgenera are present in Argentina. The subgenus *Brachistosternus* includes two large groups, one including lowland or plains species and the other mountain species from the Andes from altitudes between 2500 and 4500 m asl.

Here we report a cytogenetic study of three phylogenetically distant species of *Brachistosternus* in order to reveal possible chromosome variations in the genus. *Brachistosternus ferrugineus* (Thorell 1876) was selected as a representative of the subgenus *Ministernus*. This species is widely distributed in central and northern Argentina, eastern Bolivia, Paraguay and possibly in southwestern Brazil (Maury 1974). Two species were selected as representatives of the subgenus *Brachistosternus*, one belonging to the Andean group and the other to the plains group. *Brachistosternus montanus* Roig-Alsina 1977 (Andean group) is restricted to high-altitude areas of the Andean region (2700 to 3500 m asl) in central-western Argentina, in the provinces of Mendoza, San Juan and La Rioja (Ojanguren-Affilastro 2003a; Roig-Alsina 1977). *Brachistosternus pentheri* Mello-Leitão 1931 (plains group) is also found exclusively in Argentina, with a widespread distribution from Salta province to the southern part of Buenos Aires province. The morph of the northernmost *B. pentheri* found in the provinces of La Rioja, Catamarca and Salta (here designated as the northern morph) differs slightly from the type material by larger size and less pronounced pigmentation (Roig-Alsina & Maury 1984; Ojanguren-Affilastro 2005b). Both morphs of *B. pentheri* were analyzed cytogenetically to determine whether they also differ in karyotype.

METHODS

Specimens.—*Brachistosternus ferrugineus*: The specimens belong to two populations from an area near the center of the known distribution of the species: three males from the locality of Chepes, La Rioja province, Argentina (31°21′00″S; 66°35′60″W), and three males from the locality of San Marcos Sierra, Córdoba province, Argentina (30°46′60″S; 64°39′00″W). *Brachistosternus montanus*: Four males were obtained from the locality of Laguna Brava, La Rioja province (28°25′50″S; 69°00′31.3″W). This population has been previously mentioned under the name *B. affinis montanus* (Ojanguren-Affilastro 2003a) because it shows minor morphological differences from the typical morph of the species, which is present in San Juan and Mendoza provinces. However, we consider this population to be the same species after examining many additional specimens.

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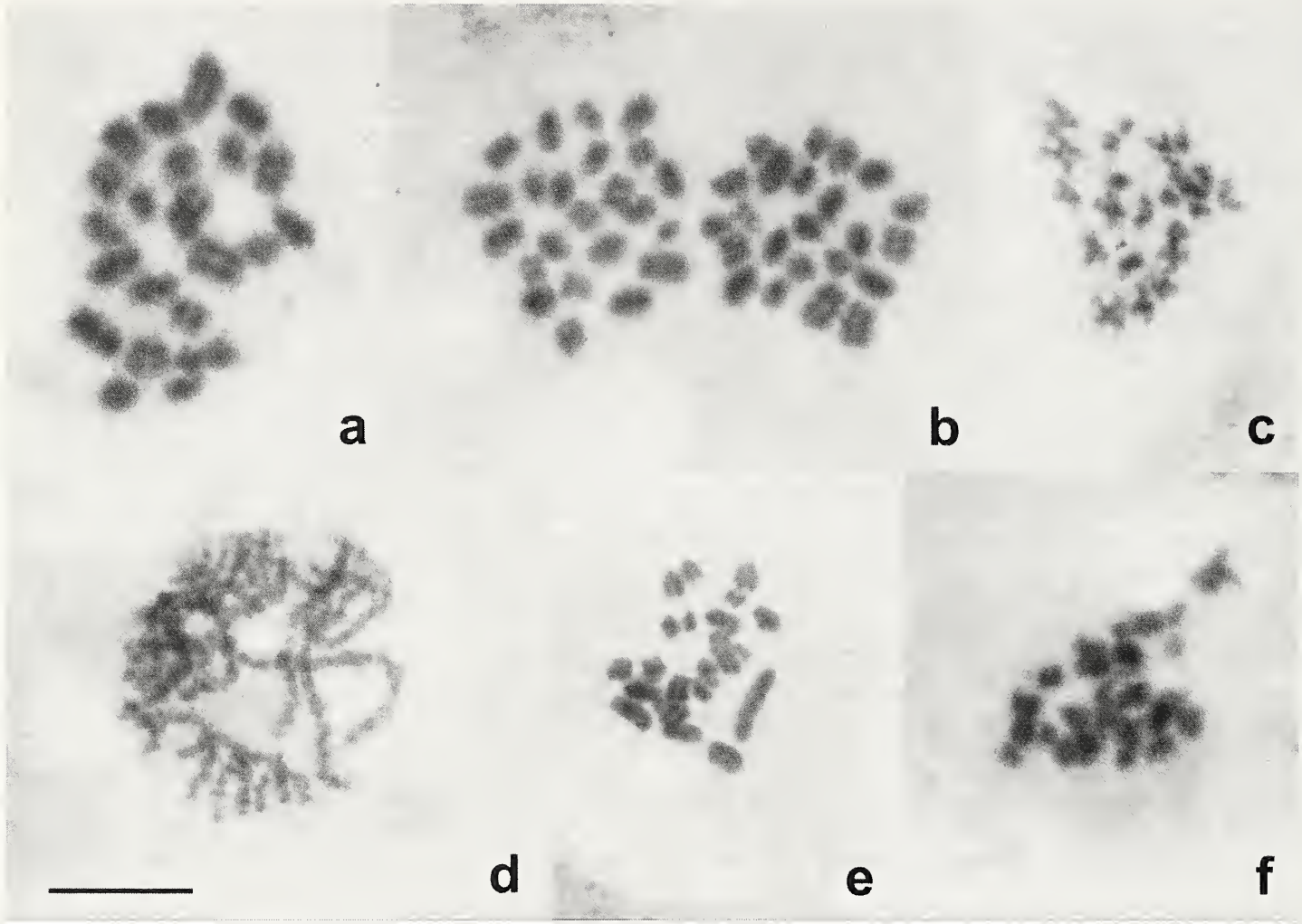

Figure 1. a–c.—*Brachistosternus ferrugineus* (n = 23). a. Late postpachytene; b. Two prometaphase I; c. Metaphase II.—d–f. *Brachistosternus montanus* ($2n$ = 46, n = 23). d. Pachytene; e. Prometaphase I; f. Prometaphase II. Scale bar: 10 μm.

Brachistosternus pentheri: Four males were obtained from the locality of Villa Unión, northern La Rioja province (29°18′00″S; 68°12′00″W) and belong to the northern morph. The typical morph was represented by three males from Chepes (southern La Rioja province) (31°21′00″S; 66°35′60″W), and one male from Oriente (coastal area of southeastern Buenos Aires province) (38°36′29″S; 60°37′07.3″W).

Cytogenetic methods.—All specimens were carried alive to laboratory and killed by cooling in a refrigerator. Their gonads were dissected in a physiological saline solution, swollen in hypotonic solution (0.56% KCl) for 10 min, and then fixed in a mixture of ethanol:chloroform:acetic acid (6:3:1). A piece of testis was placed on a slide, dissociated in a drop of 60% acetic acid with tungsten needles, and spread on the slide using a heating histological plate at approximately 45° C. Finally, the preparations were air-dried and stained with 3% Giemsa solution in water (pH = 7.4) for 10 min. Five postpachytene-prometaphase I of each cytotype of *B. peutheri* were measured to determine the meiotic karyotype. Bivalents measurements were made using the computer application Micromeasure version 3.3 (Reeves & Tear 2000). The relative length of each bivalent was calculated as a percentage of total haploid complement length (TCL). The idiogram of each cytotype was drawn on the basis of the relative percentage of each bivalent length to the TCL.

RESULTS

Subgenus *Ministernus* Francke 1985
(Figs. 1a–c; Table 1)

Brachistosternus ferrugineus has a karyotype of $2n$ = 46. No positively heteropycnotic bodies were observed at early prophase I. After pachytene, bivalents have no chiasma; homologous chromosomes lie parallel to each other, and condense gradually during prophase I and metaphase I. All bivalents are homomorphic (Figs. 1a, b). Chromosome plates of prometaphase and metaphase II consist of bibrachial (meta- or submetacentric) and monobrachial chromosomes of different sizes (Fig. 1c). No differences were observed between the specimens from the two localities.

Subgenus *Brachistosternus* Pocock 1893
(Figs. 1d–f, 2a–f, 3a–d; Table 1)

The chromosome complement of *B. montanus* consists of 46 chromosomes. At early prophase I no positively heteropyc-

Table 1.—Karyotype characteristics and collecting localities of the Bothriuridae species cytogenetically analyzed.

Species	$2n$	n	Locality	References
Bothriurus sp.	36	-	Três Lagoas, Matto Grosso, Brazil	Piza 1947
Bothriurus araguayae Vellard 1934	44	22	São Paulo, Brazil	Ferreira 1968 (sub. *B. asper araguaie*)
Bothriurus flavidus Kraepelin 1911	48	24	Buenos Aires Province, Argentina	Giacomozzi 1977
Bothriurus prospicuus Mello-Leitão 1934	50	25	Buenos Aires Province, Argentina	Giacomozzi 1977
Brachistosternus alienus Lönnberg 1898	28	14	Chubut Province, Argentina	Giacomozzi 1977
Brachistosternus ferrugineus (Thorell 1876)	46	23	San Marcos Sierra, Córdoba Province, Argentina	This work
B. ferrugineus	46	23	Chepes, La Rioja Province, Argentina	This work
Brachistosternus montanus Roig-Alsina 1977	46	23	Laguna Brava, La Rioja Province, Argentina	This work
Brachistosternus pentheri Mello-Leitão 1931	46	23	Villa Unión, La Rioja Province, Argentina	This work
B. pentheri	42	21	Chepes, La Rioja Province, Argentina	This work
B. pentheri	42	21	Oriente, Buenos Aires Province, Argentina	This work
B. pentheri	42	21	Buenos Aires Province, Argentina	Giacomozzi 1977(sub. *B. psammophilus* Maury)
Timogenes elegans (Mello-Leitão 1931)	48	24	Río Negro Province, Argentina	Giacomozzi 1977

notic bodies are present (Fig. 1d). Meiosis is achiasmatic; all bivalents are homomorphic, condensing gradually until metaphase I (Fig. 1e). Bibrachial and monobrachial chromosomes of different sizes were observed at prometaphase and metaphase II (Fig. 1f).

Morphs of *B. pentheri* showed different chromosome numbers. The karyotype of the northern morph (Villa Unión, northern La Rioja) exhibits 46 chromosomes (cytotype I) (Fig. 2), whereas karyotype of the typical morph from Chepes (southern La Rioja) and Oriente (southeastern Buenos Aires)

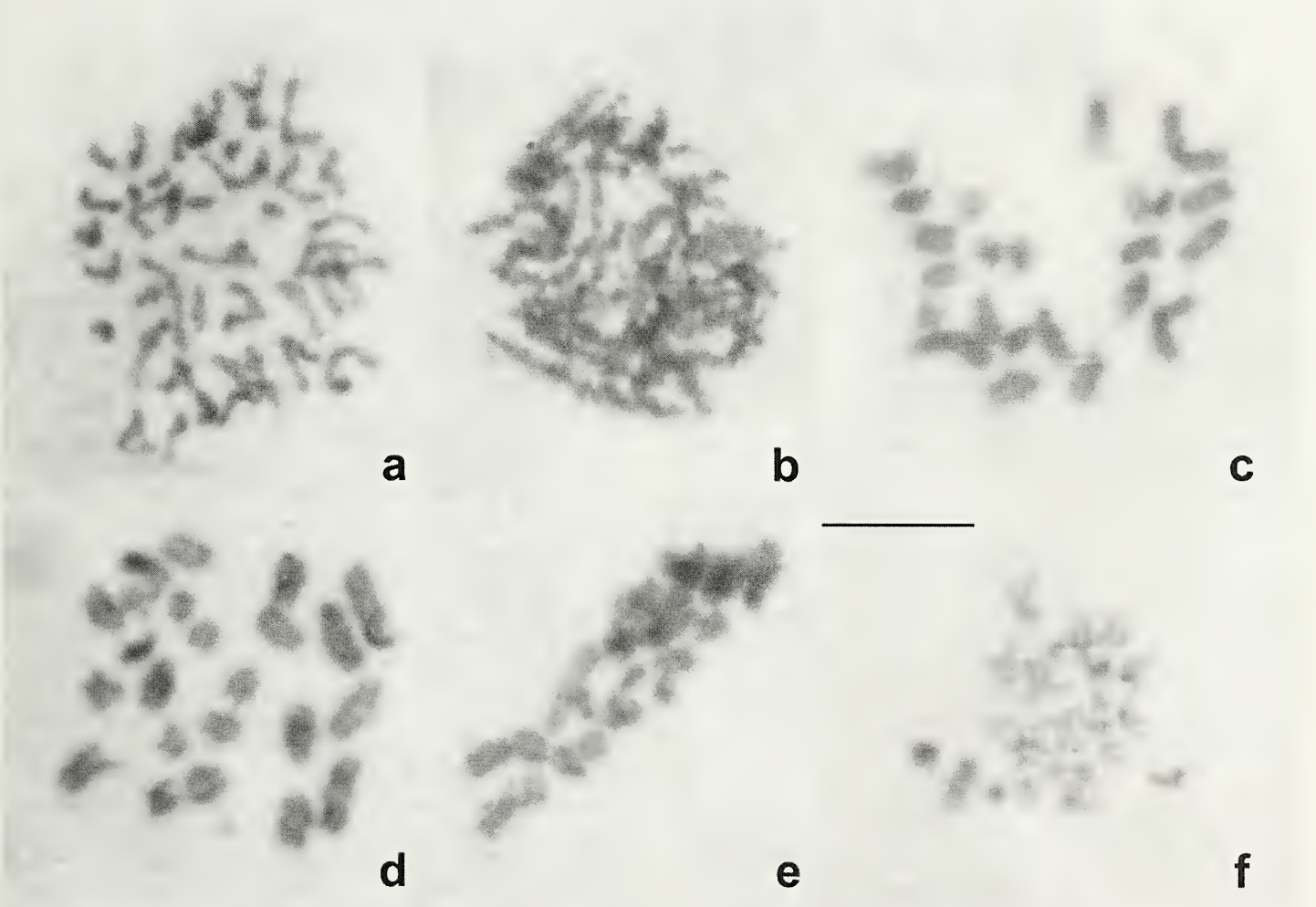

Figure 2. a–f.—*Brachistosternus pentheri* ($2n$ = 46, n = 23). a. Spermatogonial prometaphase; b. Pachytene; c. Postpachytene; d. Prometaphase I; e. Metaphase I; f. Metaphase II. Scale bar: 10 μm.

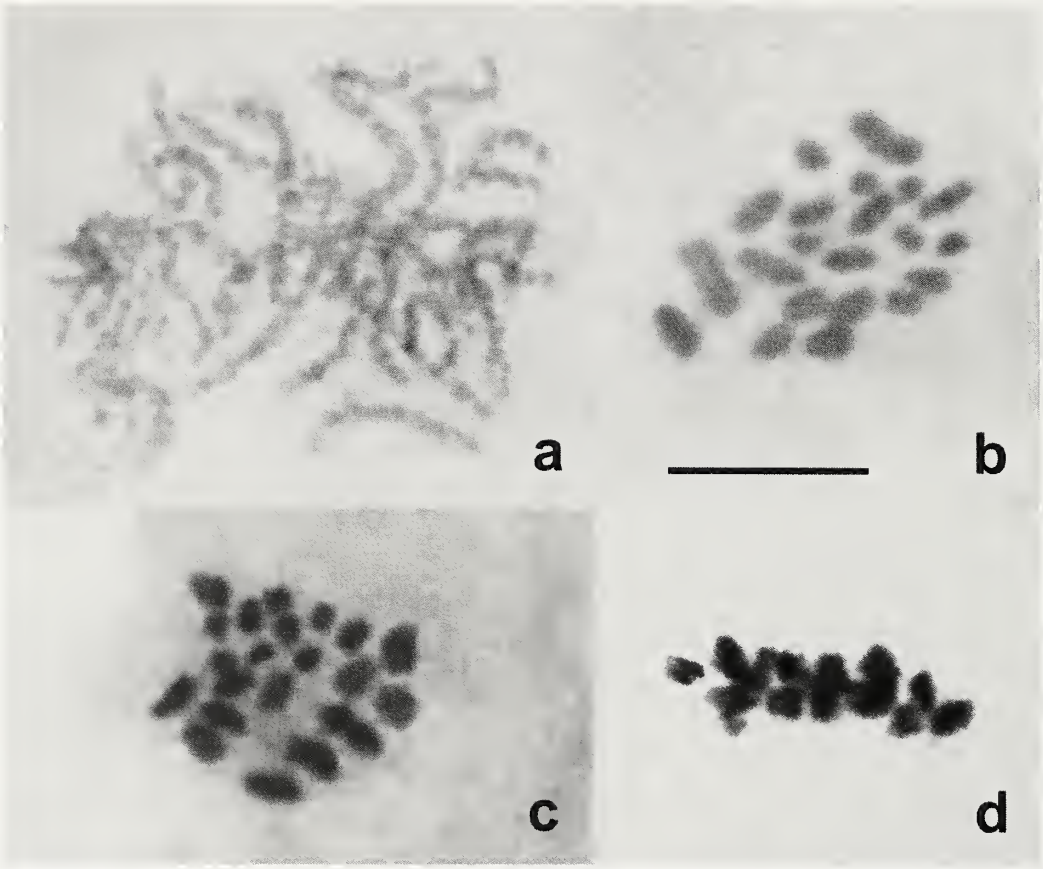

Figure 3. a–d.—*Brachistosternus pentheri* ($2n = 42$, $n = 21$). a. Pachytene; b. Late postpachytene; c. Prometaphase I; d. Metaphase I. Scale bar: 10 μm.

consisted of 42 chromosomes (cytotype II) (Fig. 3). Chromosomes of both morphs presented the same meiotic behavior. At spermatogonial prometaphases and metaphases chromosomes of different sizes were observed (Fig. 2a). At prophase I no positively heteropycnotic bodies were detected (Figs. 2b, 3a); meiosis is achiasmatic, and bivalents are homomorphic, condensing gradually during prophase I (Figs. 2c–e, 3b–d). Bibrachial (metacentric or submetacentric) and monobrachial chromosomes could be identified at metaphase II of individuals with $2n = 46$ (Fig. 2f). No metaphases II were observed in the individuals with $2n = 42$. Karyotype of *B. pentheri* cytotype I ($n = 23$) was formed by three larger bivalents of different size and the rest of the complement decreasing gradually in size (from medium-sized to small bivalents) (Fig. 4a, Table 2). Karyotype of *B. pentheri* cytotype II ($n = 21$) was formed by four larger bivalents of different size, 10 medium-sized bivalents that gradually decreased in size, and seven smaller bivalents that gradually decreased in size (Fig. 4b, Table 2).

DISCUSSION

This study focuses on cytogenetics of bothriurid scorpions of the genus *Brachistosternus*, comparing representatives of the subgenera *Ministernus* (*B. ferrugineus*) and *Brachistosternus* (*B. montanus* and *B. pentheri*). The karyotype of studied species is formed by a mixture of bibrachial and monobrachial chromosomes of different sizes. Meiotic complements of the three analyzed species of the family Bothriuridae contain no heteromorphic chromosome pair, which indicates the absence of heteromorphic sex chromosomes in males, as is also the case in *Buthus occitanus* (Amoreux 1789) and *Pandinus imperator* (C.L. Koch 1841) (Guénin 1957, 1961). Shanahan (1989a, 1989b) showed achiasmatic meiosis in males of Buthidae and Urodacidae. Our study of *Brachistosternus*, as well as that of Ferreira (1968) on *Bothriurus araguayae* Vellard 1934, reveals the presence of this derived type of meiosis also in Bothriuridae.

Our study provides the first cytogenetic analysis of *B. ferrugineus* and *B. montanus*. The karyotypes of these species consist of 46 chromosomes, a number that has not been found in the family Bothriuridae previously (Table 1). Although *B. ferrugineus* is widely distributed, there are almost no morphological differences between populations and no variation in chromosome number.

In contrast to *B. ferrugineus*, *B. pentheri* shows two different morphs (Roig Alsina & Maury 1984, Ojanguren-Affilastro 2005a), and our analysis revealed karyotypic differences between them. The northern morph of *B. pentheri* from Villa Unión (northern La Rioja) shows the same chromosome number as *B. ferrugineus*. In contrast, the karyotype of *B. pentheri* from Chepes (southern La Rioja) and Oriente (southeastern Buenos Aires) consists in 42 chromosomes. These specimens correspond morphologically to the species' holotype from Mendoza province, Argentina, and they belong to the typical morph of the species. Although the range of the

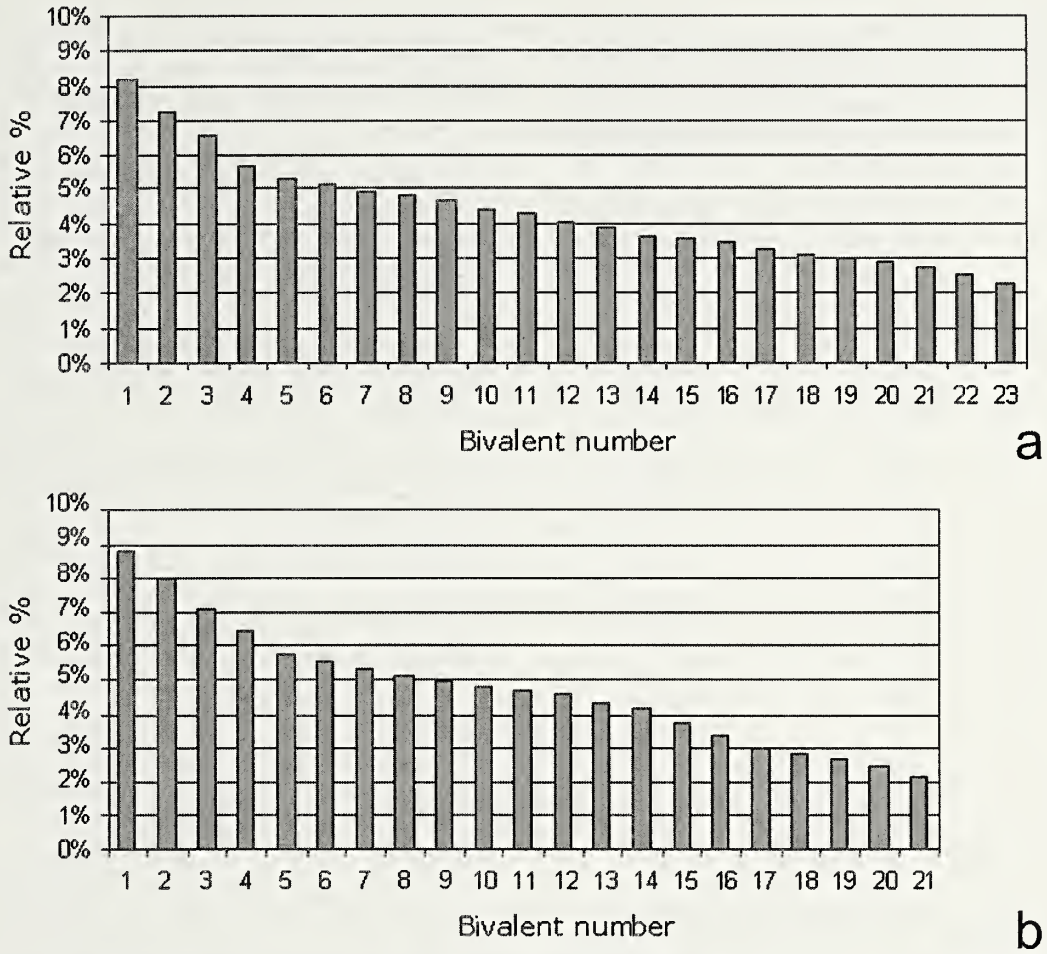

Figure 4. a–b.—Idiograms of *Brachistosternus pentheri* cytotypes. a. Cytotype I, $n = 23$; b. Cytotype II, $n = 21$.

relative lengths of the bivalents in both cytotypes was similar, three bivalent groups can be distinguished in cytotype II, but only two in cytotype I (Fig. 4). Since only 1% of *Brachistosternus* species were cytogenetically analyzed it was not possible to determine the ancestral karyotype and therefore the rearrangements that lead to the different chromosome numbers (e.g., centric or tandem fusions versus fissions). The intraspecific morphological and karyotypic variations of *B. pentheri* indicate that its marginal northern populations could be a different subspecies or even speeies. This proposal could be tested by attempting hybridization between the typical and northern morphs.

The genus *Brachistosternus* was studied cytogenetically for the first time by Giacomozzi (1977) (Tab. 1), who mentions that his specimens were collected and determined by Dr. E. Maury as *B. psammophilus* Maury 1977 and *B. alienus* Lönnberg 1898. *B. psammophilus* was considered a possible endemic species confined to coastal dunes in southern Buenos Aires province (Maury 1977). However, some years later Roig Alsina & Maury (1984) synonymized *B. psammophilus* with *B. pentheri*, a widespread species from central and northern Argentina. Therefore, the specimens studied by Giacomozzi (1977) as *B. psammophilus* should be considered *B. pentheri*. The karyotype of the specimens studied by Giacomozzi as *B. psammophilus* consisted of 42 chromosomes, like our specimens of *B. pentheri* from Oriente. Both samples of specimens belong to the same group of populations from coastal dunes of southern Buenos Aires.

On the other hand, the identity of the second species of *Brachistosternus* studied by Giacomozzi (1977) is uncertain. Maury determined these specimens as *B. alienus* (Giacomozzi 1977), but it is possible that they belong to *B. angustimanus* Ojanguren-Affilastro & Roig-Alsina 2001. At the time of Giacomozzi's investigation, most authors based the identification of *B. alienus* on the redescription by Mello-Leitão (1938, 1945), whose definition of *B. alienus* encompassed another species now known as *B. angustimanus* (Ojanguren-Affilastro 2001; Ojanguren-Affilastro & Roig-Alsina 2001). Both species are sympatric over most of their ranges, but *B. angustimanus* is more commonly found than *B. alienus* because of its larger size and higher abundance.

Brachistosternus alienus (*sensu* Giacomozzi 1977) shows the lowest chromosome number known for the genus ($n = 14$) (Giacomozzi 1977). Recent phylogenetic analyses (Ojanguren-Affilastro 2008; Ojanguren-Affilastro & Ramírez 2009) that include both morphological and moleeular data placed *B. pentheri* as the sister group of the clade (*B. angustimanus* (*B. alienus* (*Brachistosternus telteca* Ojanguren-Affilastro 2000, *Brachistosternus nultidentatus* Maury 1984))). Therefore, the assessment of whether the low chromosome number is a

Table 2.—Relative lengths (RL) of bivalents of *Brachistosternus pentheri* cytotypes.

	RL (%)	
Bivalent number	Cytotype I (n=23)	Cytotype II (n=21)
1	8.17	8.78
2	7.22	7.99
3	6.57	7.14
4	5.70	6.42
5	5.34	5.79
6	5.13	5.57
7	5.00	5.39
8	4.85	5.12
9	4.65	4.96
10	4.41	4.83
11	4.29	4.71
12	4.08	4.58
13	3.91	4.33
14	3.68	4.17
15	3.63	3.74
16	3.48	3.36
17	3.28	3.02
18	3.11	2.81
19	3.00	2.66
20	2.89	2.46
21	2.78	2.17
22	2.53	
23	2.30	

synapomorphy of the clade or an autapomorphy of the specimens determined by Maury as *B. alienus* should be made using specimens accurately identified as *B. alienus* and *B. angustimanus*.

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Redescription of *Plesiochactas mitchelli* (Scorpiones: Euscorpiidae): a rare scorpion from Central America

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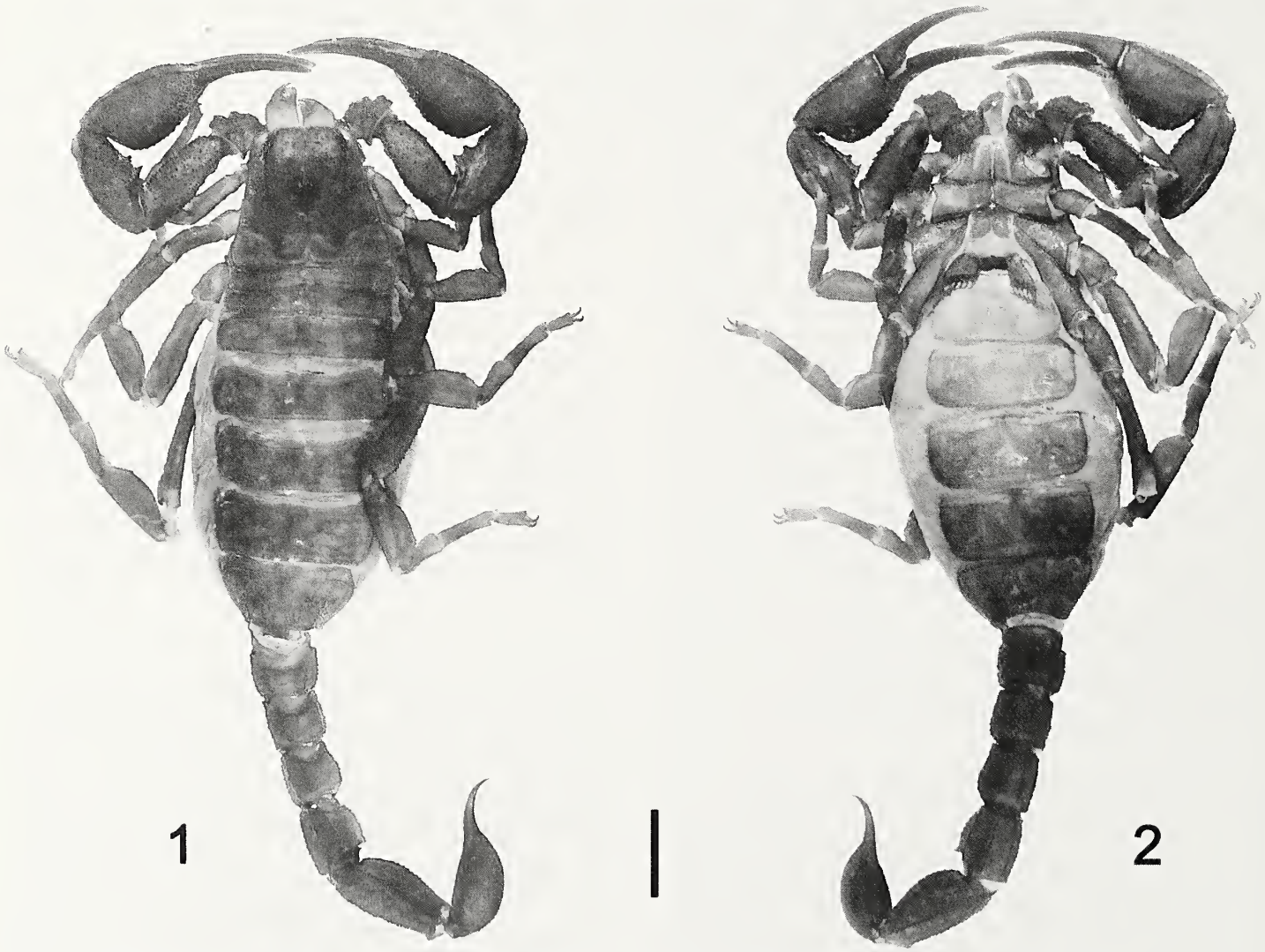
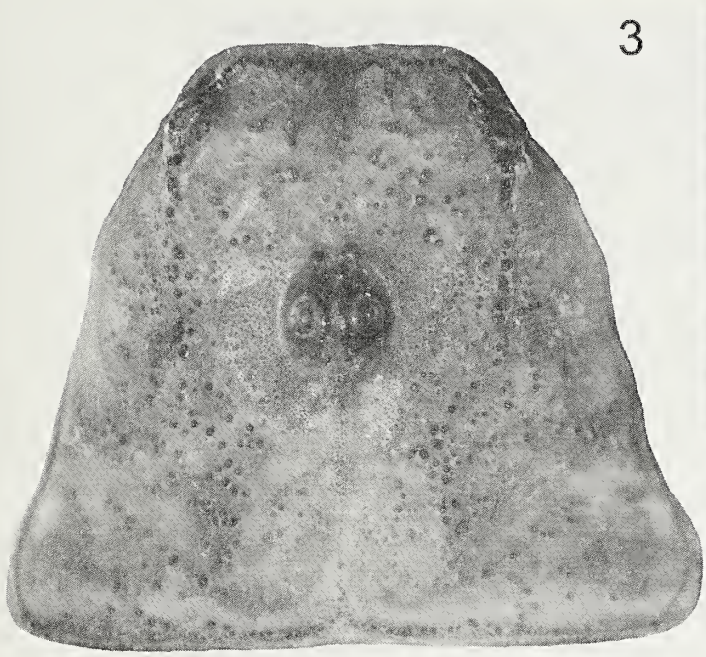
Abstract. *Plesiochactas mitchelli* Soleglad 1976 was originally described from a juvenile female collected in "Guatemala" before 1902. The species is redescribed on the basis of an adult female from a specific locality in the state of Chiapas; it is the first record of this species from México.

Keywords: Euscorpiidae, Chiapas, México, systematics

There is little information available about scorpions of the genus *Plesiochactas* Pocock 1900. This genus consists of only two species: *Plesiochactas dilutus* Karsch 1881, the type species which is known from four specimens (Soleglad 1976; Soleglad & Sissom 2001), and *Plesiochactas mitchelli* Soleglad 1976. The male is undescribed for this genus, and *P. mitchelli* is known only from a single immature specimen.



Figures 1, 2.—*Plesiochactas mitchelli* female adult specimen. 1. Dorsal aspect; 2. Ventral aspect. Scale line: 5 mm.



Figures 3, 4.—*Plesiochactas mitchelli* female adult specimen. 3. Carapace; 4. Ventral aspect of sternum and pectines.

Pocock (1902) mentioned a young specimen from "Guatemala" nearly allied to *P. dilutus*. Soleglad (1976) revised the scorpion subfamily Megacorminae, with limited material available. He described *P. mitchelli* without a precise locality from the same juvenile female reported by Pocock. To this date, no additional specimens of this species have been reported in the literature. In the present paper, we redescribe *P. mitchelli* on the basis of an adult female from southeastern Chiapas, México, from a locality not too distant from the border with Guatemala (see Remarks).

METHODS

The new specimen examined for this study is lodged in the Colección Nacional de Arácnidos (CNAN), Instituto de Biología, Universidad Nacional Autónoma de México, México, D.F. Nomenclature and mensuration primarily follow Stahnke (1970), with the following exceptions: metasomal carinal terminology after Francke (1977), pedipalp carinae terminology after Acosta *et al.* (2008), trichobothrial designations after Vachon (1974), and chelal finger dentition terminology after Soleglad & Sissom (2001). Measurements were made with an ocular micrometer at 10X on a Nikon SMZ 800 stereomicroscope; drawings on the same scope with a camera lucida attached; photographs also on the same scope with a Nikon Coolpix 20 camera.

SYSTEMATICS

Family Euscorpiidae Laurie 1896
Subfamily Megacorminae Kraepelin 1899
Genus *Plesiochactas* Pocock 1900

Plesiochactas Pocock 1900:470.

Type species.—*Plesiochactas dilutus* (Karsch 1881), by monotypy.

Plesiochactas mitchelli Soleglad 1976

Plesiochactas mitchelli Soleglad 1976:251, 263, 286, 288, 294–298, Table 4, Figs. 17, 21, 86, 87, 117, 118, 121, 123–134; Soleglad & Sissom 2001:29, 58, 67, 92, Figs. 141, 196.

Plesiochactas dilutus (in part) Pocock 1902:16, 17, Table 4, Figs. 5a–f.

Type data.—GUATEMALA: Holotype juvenile female from "Guatemala (Sarg)," date unknown (deposited in Natural History Museum, London).

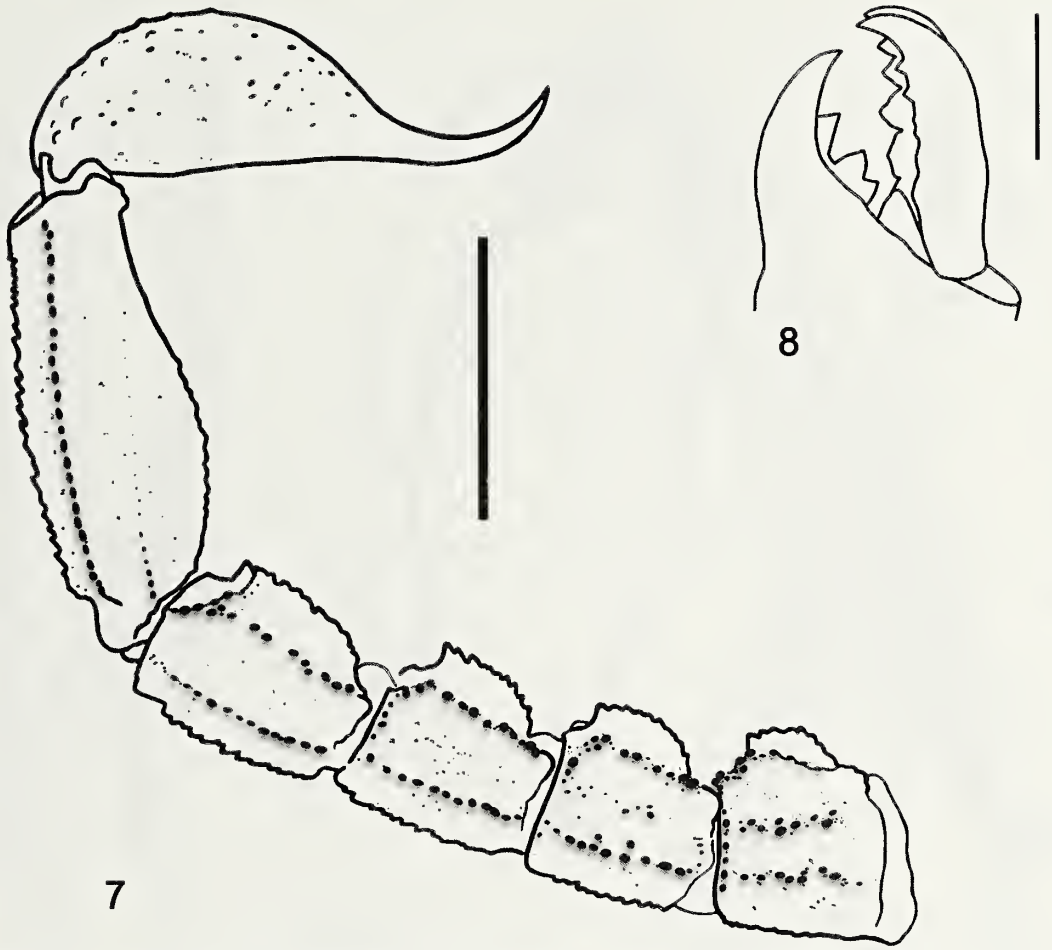
Additional specimen.—MÉXICO: *Chiapas:* 1 adult ♀, Santa Rosa, [Municipio] La Trinitaria, August 1974, [col.] A. Ramírez V. (CNAN).

Diagnosis.—Adult female 53.3 mm long. Pectines quite reduced; 5 teeth for female; fulcra absent. Median carina of sternite VII obsolete. Stigmata elongate oval. Patella with 23 trichobothria on external face: 5 each in **em** and **et** series; 8–10 ventral trichobothria in a straight row along posterior margin. *P. dilutus*, the only other species in the genus has 8–9 pectinal teeth in females, 10 in the male; the pectines have well developed fulcra; and median carina of sternite VII weak to moderately strong, smooth.

Description of adult female (Figs. 1, 2).—*Coloration*: uniformly orange-brown, darkening to reddish brown in carapace, pedipalps, metasomal segments and telson. *Prosoma*: carapace 1.2 times wider than long, densely covered with random mixture of large, medium, and small granules, anterior margin straight. Lateral eyes, two per side, well developed, posterior eye slightly smaller. Median ocular tubercle very well developed and prominent, one-fifth width of carapace at that point; placed on anterior two-fifths of carapace (Fig. 3). Sternum wider than long with some granules distally, deep median groove (Fig. 4). *Mesosoma*: tergites rough, minutely and densely granulose, with larger,

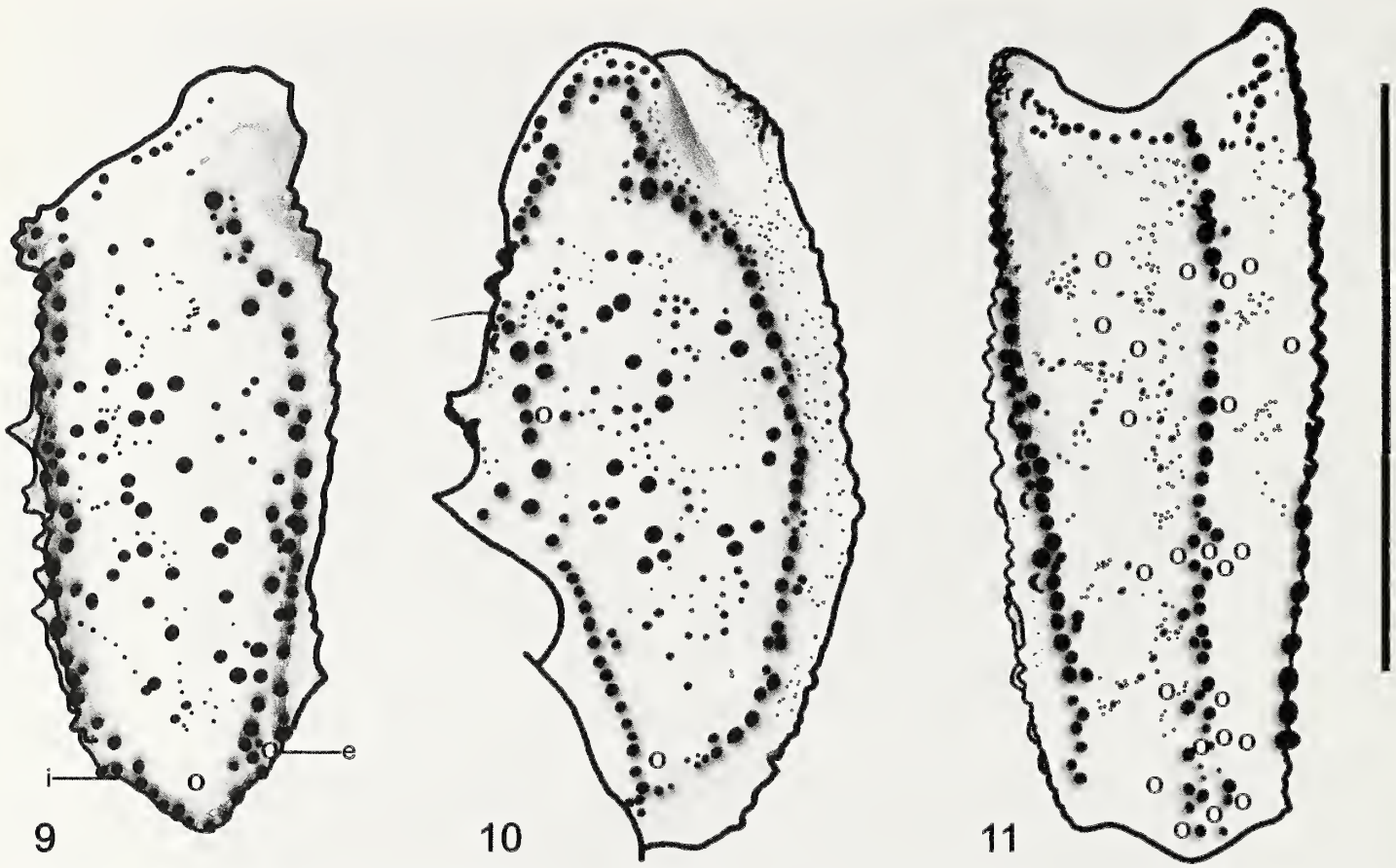
Figures 5, 6.—Stigmata of *Plesiochactas mitchelli* female adult specimen. 5. Stigmata closed; 6. Stigmata open.

sparse granules on posterolateral regions. Median carina weak to vestigial in all tergites. Tergite VII with two pairs of crenato-granulose carinae. *Genital operculum*: relatively small, subtriangular, with complete median longitudinal membranous connection. *Pectines* (Fig. 4): reduced, three marginal and one middle lamellae well defined; fulcra absent; pectinal teeth 4 times longer than wide, sensorial areas present distally, on $^{1}/_{3}$ to $^{1}/_{2}$ of tooth; tooth count 5-5. *Sternites*: III–VI lustrous; VII with a pair of vestigial lateral carinae, hinted by some granules; median carina obsolete. *Stigmata*: reniform



Figures 7, 8.—*Plesiochactas mitchelli* female adult specimen. 7. Lateral aspect of metasomal segments and telson, Scale line: 5 mm; 8. Ventral aspect of left chelicerae. Scale line: 1 mm.

elongate, with a distinctly sclerotized lid hinged distally, which opens inward to the book lung cavity (Figs. 5, 6). *Metasoma*: intercarinal spaces with sparse granules. Segments I–II wider than long, III as long as wide and IV longer than wide. Dorsolateral and lateral supramedian carinae crenulated to serrated. Posterior spines on dorsal carinae weakly developed, gradually stronger on distal segments (Fig. 7). Lateral inframedian carinae on I, granulose and complete; on II with few granules posteriorly, and on III–IV obsolete. Ventrolateral carinae on I–II, granulose; on III–IV crenulated to serrated. Ventral median carina on I weak, granulose; on II–IV crenulated to serrated. Segment V: dorsolateral and ventrolateral carinae crenulated to serrated; lateral median carina weak, indicate only on proximal region; ventral median carina, serrated; anal arc with subtle granulation. *Telson*: segment elongated, aculeus-vesicle juncture not sharply defined. Vesicle dorsally smooth, lateral and ventrally covered with low, rounded granules. Aculeus slightly curved. Subaculear tubercle/spine absent. *Chelicera*: movable finger with two subdistal denticles on dorsal edge; inferior margin with 2–4 small denticles; without serrula (Fig. 8). *Pedipalps*: dorsal face of patella and femur with scattered granules; ventral face smooth to rough. Femur orthobothriotaxic (Fig. 9): 2.6 times longer than wide; retrodorsal, prodorsal and proventral carinae, strong and crenulated; retroventral carina present only on proximal region, strong and granulose; retrolateral face with granulose to serrated median carina; prolateral face with a median carina indicate by some longer spiniform granules. *Patella* neobothriotaxic (Figs. 10, 11): retrolateral face with 23 trichobothria (5 et, 4 est, 5 em, 2 esb and 7 eb); ventrally on right side with 9 v trichobothria, left with 10 v; 2.3 times longer than wide; all carinae crenulated to serrated; retrolateral aspect with well developed median carina, crenulated; proximal projection of prolateral face with one medium and one small spine. *Chela*: Orthobothriotaxic (Figs. 12, 13): movable finger shorter than carapace and slightly shorter than fifth metasomal segment. Digital and retrolateral secondary carinae strong, crenulated; dorsal secondary and prodorsal carinae weak, granulose; retroventral carina very strong and crenulated; proventral carina weak, crenulated; ventral median carina vestigial, only present and granulose proximally; prolateral median carina crenulated. Intercarinal spaces with sparse granules. Chelal finger dentition, based on right movable finger (Fig. 14): median denticle row flanked by groups of one outer denticle plus two inner denticles; in addition, the median denticle corresponding to each group is slightly enlarged, resulting in transverse rows of four denticles each, which divide the median denticle row into distinct subrows. Distally there are no accessory denticles; basal to distal group 3 inner accessory denticles appear, and basal to distal



Figures 9–11.—*Plesiochactas mitchelli* female adult specimen. 9. Dorsal aspect of pedipalp femur; 10. Dorsal aspect of pedipalp patella; 11. Retrolateral aspect of pedipalp patella. Scale line: 5 mm.

group 5 outer accessory denticles are present. Basal to distal group 6 a poorly defined "double row" formed by the median denticle row and the inner accessory denticles can be discerned. *Measurements*: (in mm; L = length, W = width, D = depth). Total L 53.3; carapace L/W 8.0/9.3; mesosoma L 17.2; metasoma L 19.4: segment I L/W/D 2.3/3.8/3.1, segment II L/W/D 2.6/3.3/3.0, segment III L/W/D 3.0/3.2/3.1, segment IV L/W/D 4.0/3.0/3.1, segment V L/W/D 7.5/2.7/2.8. Telson L 8.7: vesicle L/W/D 5.1/3.0/2.6; pedipalp L 26.7: femur L/W 6.6/2.5, patella L/W 6.8/3.0, chela L/W/D 13.3/4.0/4.3, movable finger L 7.0, fixed finger L 5.9.

Remarks.—Francis Charles Sarg (whom we presume collected the holotype) was born in Guatemala; in the 1880s he lived in Cobán and around 1902 he owned a farm called "El Chicabal" in Quetzaltenango. It is possible the holotype was collected in or near that farm.

There are four settlements called Santa Rosa in the Municipio of La Trinitaria, Chiapas, and we do not know from which of them the specimen was collected; thus we can not provide geographical coordinates or elevation for the specific location. Quetzaltenango, Guatemala, is approximately 100 km from the Municipio of La Trinitaria.

DISCUSSION

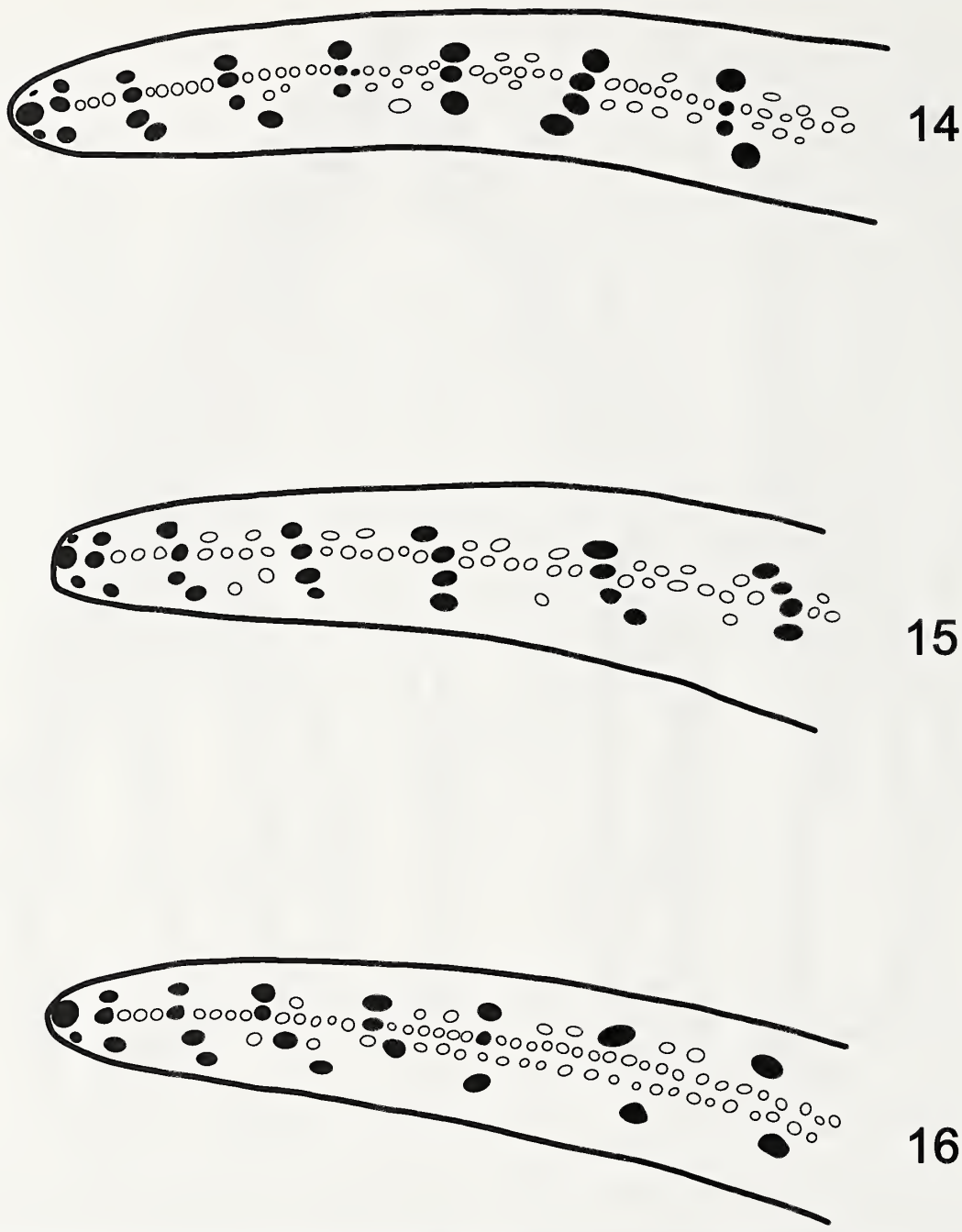
The principal difference, other than size, between the juvenile holotype and the adult female of *P. mitchelli* is the patella ventral trichobothrial number: eight in the holotype, nine and ten in the adult female specimen. This character shows similar variability in the other described species of *Plesiochactas* (*P. dilutus*: 9–12), and is also variable in species of the genus *Megacormus* Karsch 1881 (Soleglad 1976; Sissom 1994; Francke pers. obs.). The submedian and lateral carinae of sternite VII are obsolete in the holotype, and on the adult female a pair of vestigial lateral carinae are present, and the submedian carinae are also obsolete. One somewhat large denticle on the distal aspect of the ventral edge of the movable cheliceral finger of the holotype, the adult female has two to four spaced denticles. The movable finger of the pedipalp chela on the adult female is shorter than the carapace and slightly shorter than the fifth metasomal segment; in the holotype it is slightly shorter than the carapace, and longer than segment V of the metasoma. Several differences of mesosomal, metasomal, and pedipalpal carinae between the holotype and the adult female are ascribable to ontogenetic changes. Both the presence or absence of fulcra on the pectines, and the pectinal tooth counts on females, are diagnostic characters that separate the two species of *Plesiochactas*. Also, the median carina on sternite VII is weak to moderate and smooth on *P. dilutus*, and it is smooth and vestigial to obsolete on *P. mitchelli*.

The taxonomic status of this genus and its species remains uncertain. Originally, *Plesiochactas* was separated from *Megacormus* by the presence of distinct fulcra in the pectines,

Figures 12, 13.—*Plesiochactas mitchelli* female adult specimen. 12. Retrolateral aspect of right pedipalp chela; 13. Dorsal aspect of right pedipalp chela. Scale line: 5 mm.

but *P. mitchelli* lacks the pectinal fulcra. *Plesiochactas dilutus* and *P. mitchelli* share having five trichobothria on the external terminal series of the patella, as opposed to only 3–4 on *Megacormus,* and on that basis *P. mitchelli* was placed in *Plesiochactas* by Soleglad (1976). However, the number of trichobothria on that region of the patella is quite variable in the family Euscorpiidae Laurie 1896 (Soleglad & Sissom 2001), and thus the similarity between the two species currently placed in *Plesiochactas* could be a symplesiomorphy.

Soleglad (1976) and Soleglad & Sissom (2001) also indicate that *Plesiochactas* and *Megacormus* can be separated on the basis of chelal finger dentition: the former with 45+ small, inner accessory denticles, arranged row-like; the later with 35+ small outer accessory granules, scattered, irregular row-like. A direct comparison of the denticle pattern in *P. mitchelli* (Fig. 14), against those of *Megacormus gerstchi* Díaz 1966 (Fig. 15), and *P. dilutus* (Fig. 16) clearly indicate a stronger similarity between the first two, which share transverse "tetrads" along the movable finger length. In *P. dilutus*, however, the tetrads tend to disappear after distal group 3 and only enlarged inner and outer denticles are present, and a "double row" of median plus inner accessory denticles is quite distinct on the basal 2/3 of the movable finger. The second author has undertaken a revision of the tribe Megacormini Kraepelin 1899 [*Megacormus* + *Plesiochactas*], including a cladistic analysis of the phylogenetic relationships of all included taxa, which should help resolve the current uncertainty (ms. in preparation).



Figures 14–16.—Movable finger dentition of right pedipalp chela. 14. *Plesiochactas mitchelli* adult female; 15. *Megacormus gertschi* adult female from Hidalgo, Tiangüistengo; 16. *Plesiochactas dilutus* juvenile female from Oaxaca, Sta. Maria Tlahuitoltepec.

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New data on the genus *Urophonius* in Patagonia with a description of a new species of the *exochus* group (Scorpiones: Bothriuridae)

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Abstract. New data on the distribution and systematics of Patagonian species of the scorpion genus *Urophonius* Pocock 1893 are provided. A species of this genus from Península Valdés in central eastern Argentinean Patagonia, *Urophonius martinezi* new species, is described. The surface activity period of most of the species of the genus is reviewed and clearly established. A distribution map as well as a key for the Patagonian species of the genus are provided.

Keywords: Scorpiones, systematics, Neotropics, Patagonia, summer vs. winter activity

Species of the scorpion genus *Urophonius* Pocock 1893 occur in southern South America, from southern Brazil to southern Patagonia. Most species inhabit grasslands and shrub steppes, but some species have been collected in forests and even in low mountain ranges (Ojanguren-Affilastro 2005). According to Prendini (2000, 2003), the genus shows an intermediate position in the phylogeny of the family Bothriuridae, being the sister group of the genus *Cercophonius* Peters 1861 from Australia. One of the most remarkable characteristics of *Urophonius* is that most species of this genus have their surface activity period in the winter, opposite to most of the Bothriuridae, which have their surface activity period in the summer (Maury 1968, 1969, 1977, 1979; Ojanguren-Affilastro 2002, 2005). Only some species of the genus *Vachonia* Ábalos 1953 apparently share this winter activity period (López & Magnanelli 2002; Ojanguren-Affilastro 2005); however, the information available on this genus is still scarce.

In a revision of *Urophonius*, Acosta (1988) has separated the species of this genus into three different groups of species: *U. brachycentrus*, *U. granulatus*, and *U. exochus* groups. The first two groups were formerly included in a single group (group B, Maury 1973), whereas *exochus* group corresponds to group A of Maury (1973), which also corresponds to the original definition of genus *Iophorus* Penther 1913.

Most of the information we have on this genus concerns species of the *brachycentrus* group (Ábalos & Hominal 1974; San Martín & Gambardella 1974; Maury 1977; Acosta 1999), and information on members of the *exochus* and *granulatus* groups is still scarce (San Martin 1965; San Martín & Cekalovic-Kuschevich 1968; Maury 1979; Cekalovic-Kuschevich 1981; Acosta 2003). The species of the *exochus* group have only been collected in Argentina from southern Patagonia to the central part of the country. Only three species have been described in this group, *Urophonius exochus* (Penther 1913), *Urophonius nahuidensis* Maury 1973, and *Urophonius eugenicus* (Mello-Leitão 1931); all of these species have been redescribed with modern standards in a recent monograph on the Argentinean scorpion fauna (Ojanguren-Affilastro 2005). Besides these species, several specimens of this group have already been cited from a wide area of central and southern Patagonia; however, in most cases they were juveniles or females without clear diagnostic characters for assigning them to a species; so our previous information about this genus in Patagonia is scarce and fragmentary. Recently we have collected additional material of this genus that allowed us to clarify, at least partially, some aspects of the systematics of this group. In this contribution we describe a new species of the *exochus* group; we provide information about the surface activity period of most species of this genus, as well as a key and a distribution map of the Patagonian species of *Urophonius*.

METHODS

Descriptive terminology follows Mattoni & Acosta (2005) for the hemispermatophores, Vachon (1974) for the trichobothria, and Francke (1977) for the metasomal earinae, abbreviated as follows: DL: dorsolateral; LIM: lateral inframedian; LSM: lateral supramedian; VSM: ventral submedian; VL: ventrolateral; VM: ventromedian. We followed Francke (1977) for the pedipalp carinae, abbreviated as follows: DI: dorsal internal; DE: dorsal external; VI: ventral internal; VE: ventral external; D: digital; E: external; V: ventral; VM: ventral median; DM: dorsal marginal; DS: dorsal secondary. Abbreviations of collections are as follows: AMNH: American Museum of Natural History (New York, USA); CDA: Catedra de Diversidad Animal I (Universidad de Córdoba, Córdoba, Argentina); MACN-Ar: Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia", (Buenos Aires, Argentina); MHNC: Museo de Historia Natural, Facultad de Ciencias Biológicas, Universidad San Antonio Abad del Cusco (Cusco, Peru); CENPAT: Centro Nacional Patagónico (Puerto Madryn, Argentina); MZUC: Museo de Zoología de la Universidad de Concepción, (Concepción, Chile). Other abbreviations: NPA-PV: Natural Protected Area Península Valdés. Illustrations were produced using a Leitz Wetzlar stereomicroscope and camera lucida. Photographs where taken using a Digital Camera (Nikon DXM 1200) attached to a stereomicroscope (Nikon SMZ 1500), the focal planes composed with Helicon Focus 3.10.3 (Online at http://

helicon.com.ua/heliconfocus/). Measurements, taken using an ocular micrometer, were recorded in mm. Scorpions where collected manually by ultraviolet collection at night, and by pitfall traps in Natural Protected Area Península Valdés (NPA-PV). Traps were placed in shrubby steppes with 40–60% vegetation cover, where the shrub *Chuquiraga avellanedae* Lorentz and the grass *Stipa tenuis* Philippi were the most representative species. The traps used were open plastic containers, 11 cm in diameter and 12 cm depth, with 300 cm^3 of 30% propylene glycol; the traps were neatly buried in the soil near *Ch. avellanedae* bushes. Trap contents were collected after 15 days, fixed in 70% ethyl alcohol, and taken to the laboratory for specimen identification.

RESULTS

Family Bothriuridae Simon 1880
Genus *Urophonius* Pocock 1893

Urophonius Pocock 1893:100–101.

Type species.—*Urophonius iheringi* Pocock 1893, by original designation.

Urophonius martinezi new species
Figs. 1, 2, 6–11, 13, 18, 20; Table 1

Urophonius sp. grupo *exochus*: Ojanguren-Affilastro 2002:185, 186 ("ejemplares del grupo *exochus* provenientes de Península Valdés"); Ojanguren-Affilastro 2005:145, 146 ("escorpiones provenientes de Península Valdés [...] muy relacionados con *U. eugenicus*").

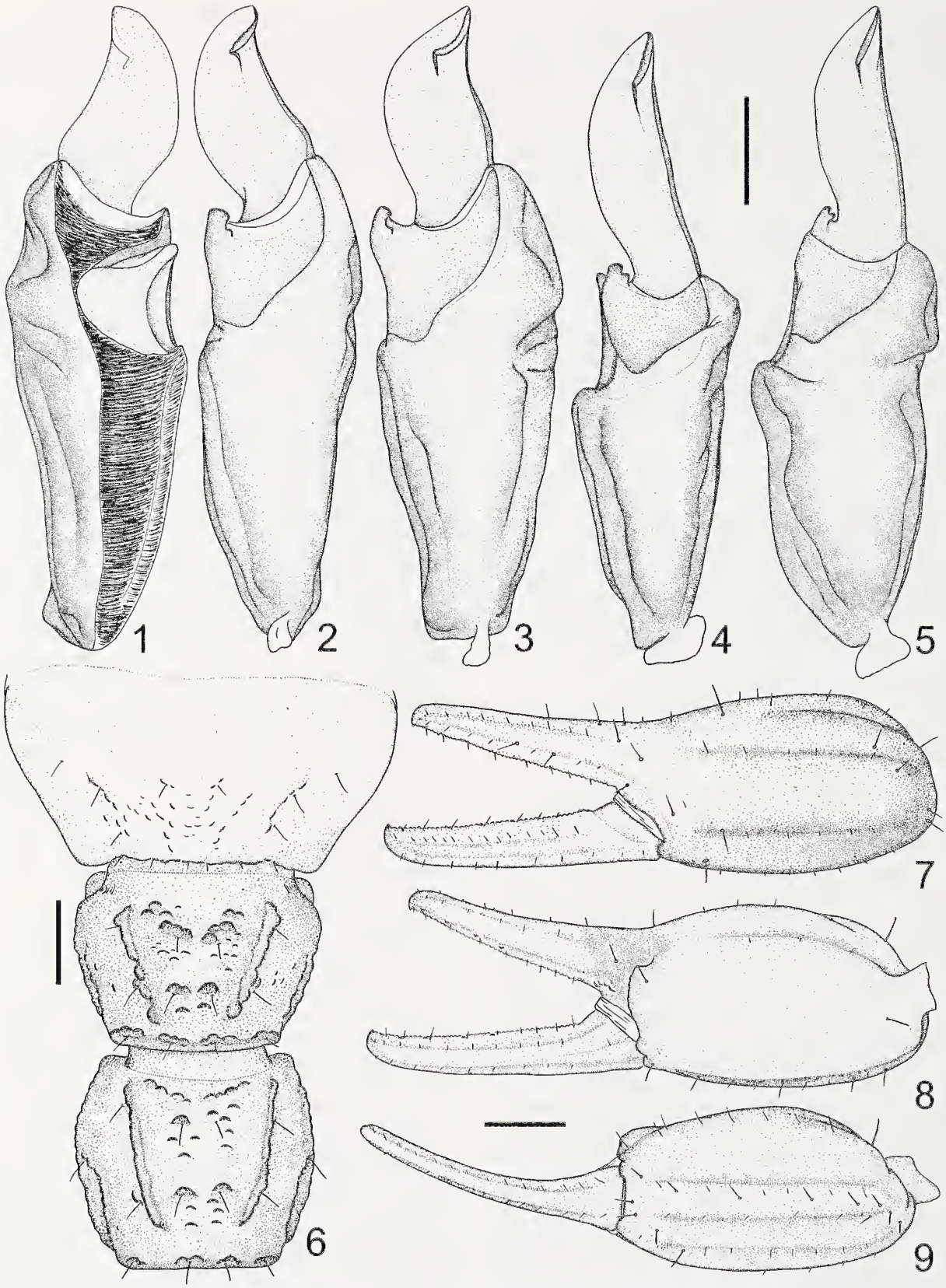
Type series.—ARGENTINA: *Chubut*: Holotype male (MACN-Ar 15808) 20 km N from Puerto Madryn (42°32′49.4″S, 64°48′25″W), 6–10 June 2008, Ojanguren-Affilastro, Martínez & Cheli.

Paratypes: ARGENTINA: *Chubut Province*: 2 ♂, Lab. Vida Silvestre, San José Gulf (42°27′51.01″S, 64°29′57.15″W), 28 October 1970, J. Dacinde (MACN-Ar 15805); 1 ♂, 2 ♀, 4 juveniles, La Falsa, Península Valdés (42°13′26.6″S, 63°51′45.3″W), May–July 2005, G. Cheli (MACN-Ar 15806); 12 ♂, 7 ♀, 12 juveniles, area around Puerto Madryn, (specimens were collected at different points along the coastal road close to Puerto Madryn: 42°49′10.8″S, 64°54′00.1″W; 42°48′02.7″S, 64°57′17.3″W; 42°39′52.7″S, 64°59′37.8″W; 42°36′57.8″S, 64°51′47.4″W; 42°32′49.4″S, 64°48′25″W), 6–10 June 2008, Ojanguren-Affilastro, Martínez & Cheli (MACN-Ar); 1 ♂, 1 ♀, 1 juvenile, same data, (CDA); 1 ♂, 1 ♀, 1 juvenile, same data, (MHNC); 1 ♂, 1 ♀, 1 juvenile, same data, (AMNH).

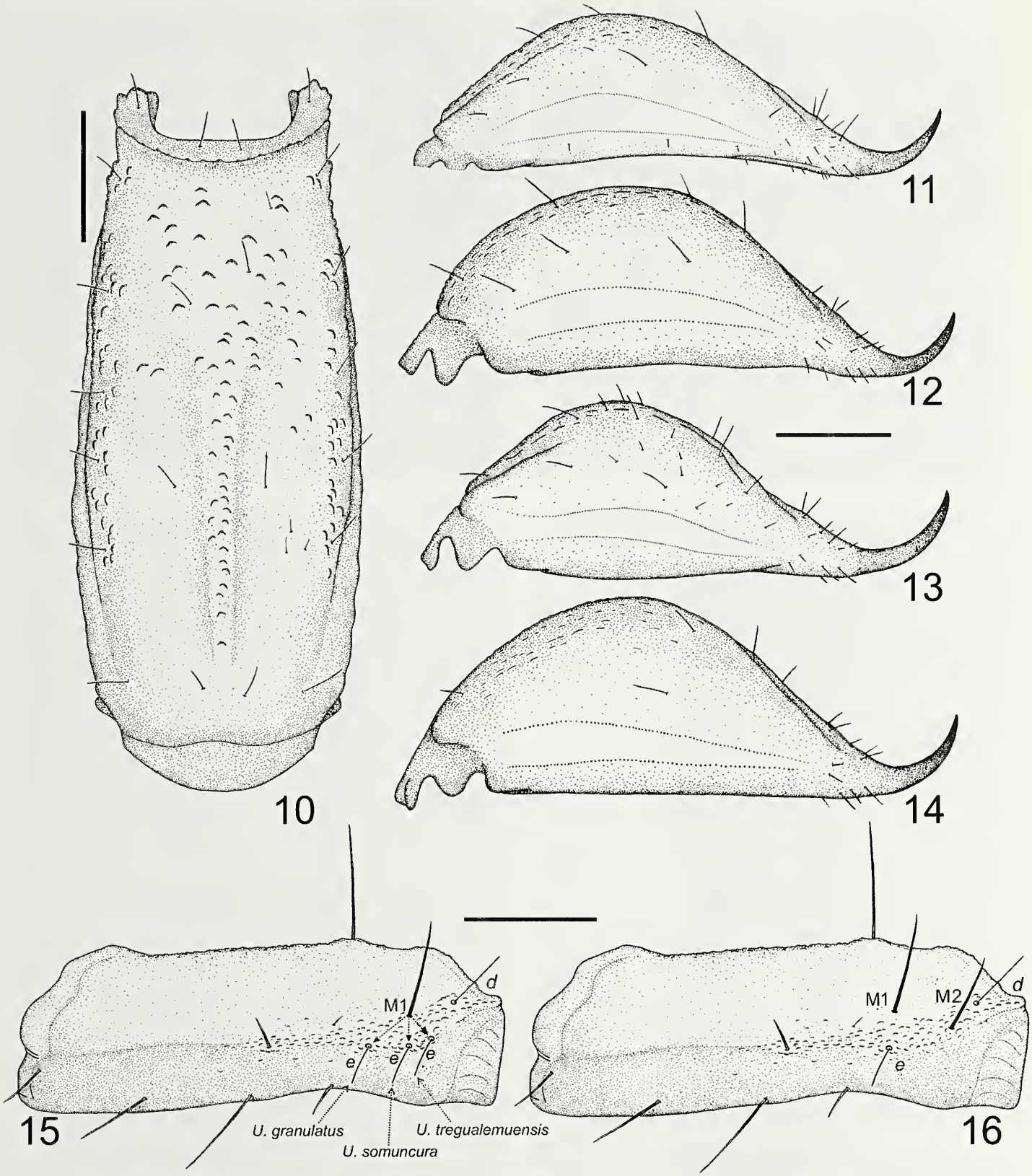
Etymology.—This species is named after the entomologist Juan José Martínez (MACN, CONICET), who has collected most of the type material of this species.

Diagnosis.—*Urophonius martinezi* is most similar to *U. eugenicus* from southern Patagonia. Both species can be separated by the shape of the telson, which is more globose in *U. eugenicus*, with its vesicle more expanded towards the sting and the posterior part of the telson (Figs. 11–14). This difference is more conspicuous in males than in females, because the telson of *U. martinezi* males is dorsoventrally compressed (Fig. 11), such that males of both species can be separated by means of the length/height ratio of the telson: *U. martinezi* 3.10–3.52, $n = 20$, mean = 3.28; *U. eugenicus* 2.54–3.03, $n = 18$, mean = 2.85; (in females the difference in shape is not reflected by the difference in this ratio). We have not found any male specimen in which this ratio overlaps; however, the extremes of variation are very close, so this possibility cannot be discounted. There are other differences between these species. *Urophonius eugenicus* is more densely pigmented than *U. martinezi*: the carapace of *U. eugenicus* has a broad irregular stripe between the lateral eyes and the ocular tubercle (Fig. 17), whereas in *U. martinezi* this stripe does not exist, or it is reduced to some isolated spots (Fig. 18). The ventral surface of tergite V and metasoma of *U. eugenicus* is more densely granular than in *U. martinezi*. In tergite V of *U. eugenicus* females there are two well developed VL carinae, and between them there are abundant coarse granules, with the VSM carinae almost indistinguishable; whereas in *U. martinezi* between the VL carinae there are some tiny scattered granules and two poorly developed VSM carinae (Fig. 6).

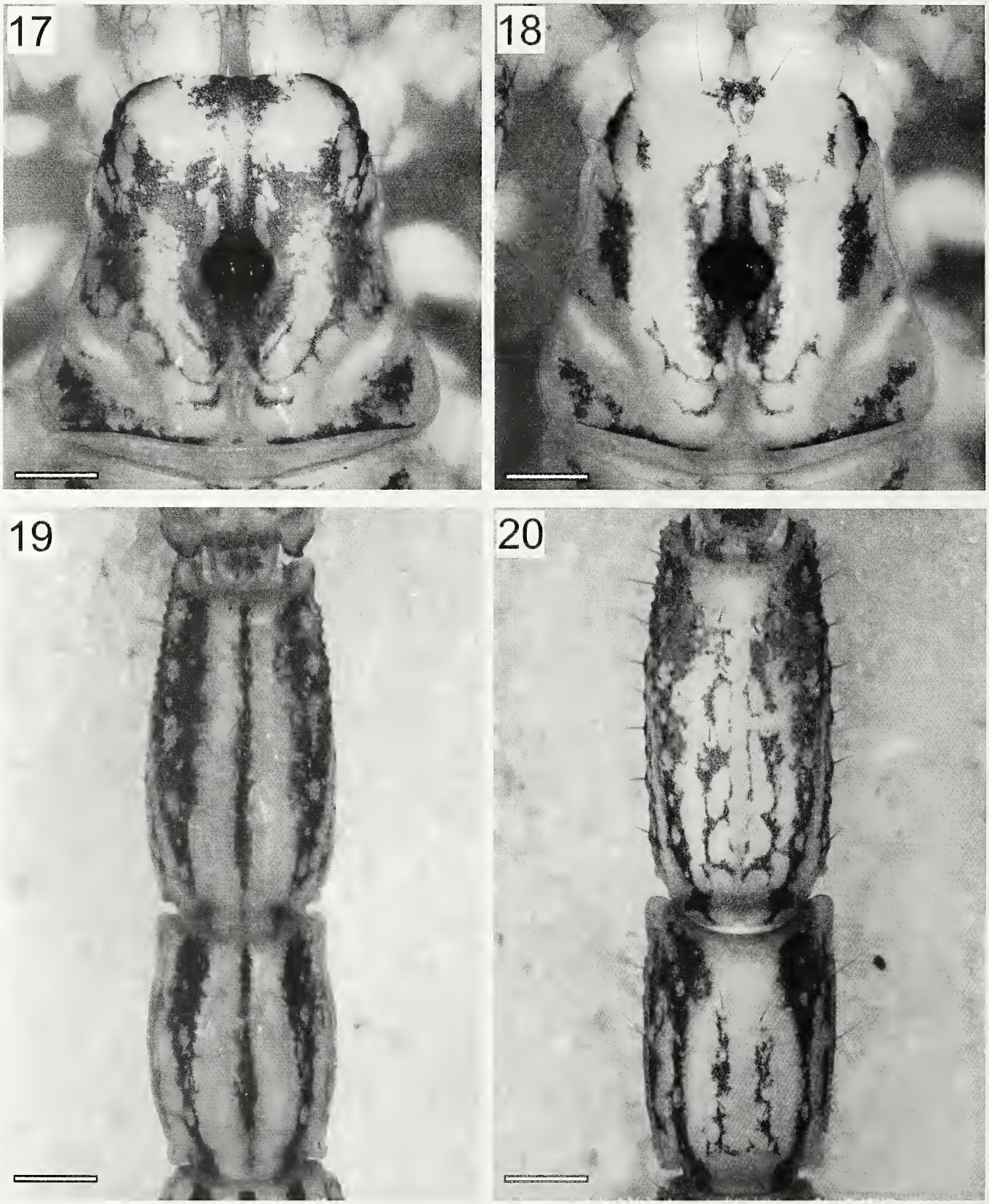
Description.—*Color*: General color yellowish, with dark brown spots of pigment. *Carapace*: anterior margin with a little dark spot in its median area, around the median notch; ocular tubercle and area around the lateral ocelli dark brown; and in the median part of the carapace there are two irregular dark stripes that surround the ocular tubercle, the postocular furrow, and the anterior longitudinal sulcus. With two lateral irregular dark spots that connect with the lateral eyes, and with two little posterolateral dark spots (Fig. 18). *Chelicerae*: fixed finger with reticular pigment, especially near the articulation with the movable finger; movable finger densely pigmented in the external margin. *Tergites*: with two lateral and two paramedian dark stripes, the lateral stripes are very narrow and occupy almost the entire length of the segment, from the posterior edge, almost reaching the anterior margin; the paramedian stripes are triangular shaped, and extend from the posterior margin to the median part of the segment. Sternites, sternum, genital opercula and pectines unpigmented. *Metasoma*: segment I: ventral surface with two VL stripes extending the entire length of the segment and two VSM stripes poorly developed and barely visible; lateral surface slightly pigmented over the LSM carina; the rest of the segment unpigmented. Segments II–IV: ventral surface with two VL and two VSM stripes poorly marked but extending the entire length of the segment; lateral surface with a dark stripe over the LSM carinae, which is thicker near the posterior margin and fuses with the VL stripes; dorsal surface with a little median triangular spot. Segment V: ventral surface with two VL and two VSM stripes extending the entire length of the segment. The VSM stripes are very diffuse and very ramified, connecting between them and with the VL stripes (Fig. 20); lateral surface with a poorly marked and very ramified stripe over the line of LSM setae; dorsal surface slightly pigmented in the median part and on the lateral margins. *Telson*: vesicle with dorsal surface unpigmented in its median area. In males, the telson gland is light yellow; slightly pigmented near the lateral margins; ventral and lateral surfaces densely pigmented; aculeus dark brown. *Legs*: femur and patella densely pigmented on the internal surface and near the articulation, the rest unpigmented. *Pedipalps*: femur and patella slightly pigmented near the articulations and on the dorsal and external surfaces, the rest unpigmented; chela, with six longitudinal stripes over the DI, DM, DS, D, E, V and VM carinae.



Figures 1–9.—1, 2, 6–9. *Urophonius martinezi* new species: 1. Left hemispermatophore, internal aspect; 2. Left hemispermatophore, external aspect; 6. Sternite V and metasomal segments I and II, female, ventral aspect; 7. Left pedipalp chela, female, external aspect; 8. Right pedipalp chela, male, internal aspect; 9. Right pedipalp chela, male, ventral aspect. 3. *Urophonius eugenicus*, left hemispermatophore, external aspect. 4. *Urophonius exochus*, left hemispermatophore, external aspect. 5. *Urophonius mahuidensis*, left hemispermatophore, external aspect. Scale bars: 1 mm.



Figures 10–16.—10, 11, 13. *Urophonius martinezi* new species: 10. Metasomal segment V, male, ventral aspect; 11. Telson, male, lateral aspect; 13. Telson female, lateral aspect. 12, 14. *Urophonius eugenicus*: 12. Telson male, lateral aspect; 14. Telson female, lateral aspect. 15. Retrolateral aspect of a stereotyped left pedipalp femur of a *Urophonius* belonging to the *granulatus* group, showing the relative position of *e* trichobothria respect to M1 macroseta in the different species of the group. 16. Retrolateral aspect of a stereotyped left pedipalp femur of a *Urophonius* belonging to the *brachycentrus* group. Scale bars: 1 mm.



Figures 17–20.—17. *Urophonius eugenicus*, carapace, dorsal aspect. 18. *Urophonius martinezi*, carapace, dorsal aspect. 19. *Urophonius granulatus*, metasomal segments IV and V, ventral aspect. 20. *Urophonius martinezi*, metasomal segments IV and V, ventral aspect. Scale bars: 1 mm.

Table 1.—Measurements of a male and a female paratype of *Urophonius martinezi* (MACN-Ar 15807).

Measurements (mm)	*Urophonius martinezi* new species	
	Male paratype	Female paratype
Total length	32.24	33.23
Carapaee, length	4.04	4.05
Carapace, anterior width	2.83	2.91
Carapaee, posterior width	4.85	4.28
Mesosoma, total length	7.27	11.48
Metasoma, total length	15.27	12.85
Metasomal segment I, length/width/height	2.02/2.59/1.94	1.70/2.67/2.02
Metasomal segment II, length/width/height	2.42/2.51/1.94	2.10/2.59/2.01
Metasomal segment III, length/width/height	2.59/2.51/1.94	2.42/2.51/1.95
Metasomal segment IV, length/width/height	3.39/2.34/1.86	2.83/2.42/1.78
Metasomal segment V, length/width/height	4.85/2.18/1.65	3.80/2.42/1.70
Telson, length	5.66	4.85
Vesicle, length/width/height	4.44/2.34/1.78	3.88/2.34/1.78
Aculeus, length	1.21	0.97
Femur, length/width	3.88/1.21	3.56/1.21
Patella, length/width	3.72/1.37	3.64/1.41
Chela, length/width/height	7.03/2.26/2.66	6.30/1.86/2.18
Movable finger, length	3.55	3.35

Morphology: Measurements of a paratype male and a paratype female (MACN-Ar 15807) are recorded in Table 1. Total length in males 28.50–36 mm (n = 14, mean = 32.25), 29–39 in females (n = 12; mean = 34.60). *Carapace*: tegument slightly granular on the lateral margins, the rest smooth; anterior margin with a well developed median notch; anterior and posterior longitudinal sulci, lateral sulcus and postocular furrow well developed; ocular tubercle well developed, median eyes well developed, one diameter apart; with three lateral eyes on each side of the carapace placed in a small bulge, two of them placed in the lower part of it, aiming to the front and the lateral margins, and the third one, 20% smaller than the others, is placed in the upper part of the bulge, aiming to the posterolateral margin. *Chelicerae*: with two well developed subdistal teeth. *Tergites*: I–VI completely smooth or slightly granular near the posterior margin; VII slightly granular in the posterior half, with four longitudinal carinae (two paramedians, two laterals) marked by coarse granulation, the lateral carinae occupying almost the entire length of the segment, whereas the paramedian carinae are restricted to the posterior half of the segment. *Sternites* I–IV with smooth tegument, spiracles small and slightly elliptic; sternite V: smooth in the anterior half, posterior half slightly granular, with four longitudinal carinae poorly marked. *Metasoma*: segment I: ventral surface with two VL carinae well developed, two VSM carinae formed by scattered coarse granules diverging proximally almost forming a transversal carinae, with four ventral macrosetae (n = 10), two over each VSM carina, with four VL macrosetae (n = 10), two over each VL carina, with four distal macrosetae (n = 10) (Fig. 6); lateral surface: LIM carinae granular and well marked in the posterior three quarters of the segment, with one macroseta over the anterior third of the LIM carina, LSM and DL carinae well marked occupying the entire length of the segment; dorsal surface smooth. Segment II: similar to segment I, but the LIM carina is restricted to the second half of the segment, and there is a macroseta over the posterior third of the LSM carina. In some specimens there is also a DL macroseta. Segment III: ventral surface, VSM and VL carinae poorly marked, longitudinal, and occupying the entire length of the segment; lateral surfaces, LIM carina restricted to the posterior third of the segment, the rest similar to segment II. Segment IV: ventral surface smooth, or with poorly developed VL carinae, with four to six ventral macrosetae (n = 10; median = 6) and three or four VL macrosetae (n = 10; median = 3); lateral surface, LSM and DL carinae represented by a slight elevation of the tegument, and occupying the entire length of the segment, lateral surface with one to three LSM setae (n = 10; median = 2) and one DL macroseta (n = 10), the rest of the tegument smooth. Segment V: very elongated, ventral surface with some coarse scattered granules in the posterior third of the segment, VM and VL carinae occupying the entire length of the segment. Very close to the VL carinae there is a group of granules parallel to the lateral margin (Fig. 10) that apparently correspond to a VSM carina; with six or seven ventral macrosetae (n = 10; median =7) and five to seven VL macrosetae on each lateral margin of the segment (n = 10; median = 6); lateral surface smooth, LSM carina represented by a row of six to nine macrosetae (n = 10; median = 9), with one to three DL macrosetae (n = 10; median = 1). *Telson*: vesicle low and elongated in males, globose in females (Figs. 11, 13), ventral surface slightly granular; dorsal surface smooth, with a well developed median glandular depression in males; aculeus very short and curved. *Legs*: smooth tegument, with two well developed and symmetrical pedal spurs; telotarsi low and elongated, with a ventromedian row of hyaline setae of the same length as the VL spines, and with well developed ventrolateral spines; spinal formula typical of the group: tarsus I: 1-1 (n = 15); tarsus II: 2-2 (n = 15), tarsus III: 4-4 in most specimens (n = 13), but in some cases there is one additional external spine 4-5 (n = 2), tarsus IV: 4-5 in most specimens (n = 12), but in some specimens there is an additional internal spine 5-5 (n = 3); telotarsal ungues symmetrical, very curved. *Pectines*: number of pectinal teeth in males: 16–18 (n = 10; median = 17); in females: 15–17 (n = 10; median = 16). *Pedipalps*: femur: DE and VE carinae

extending the entire length of the segment, slightly granular in males, blunt in females; DI and VI carinae granular and well developed, extending the entire length of the segment. Patella: DI carina blunt, with some granules near the articulation with the femur, extending the entire length of the segment, VI carina granular, extending the entire length of the segment, DE and VE carinae blunt, extending the entire length of the segment, internal median carina represented by some scattered granules in the median part of the segment. Chela: slightly elongated, more robust in males, (Figs. 7, 9); on the internal surface males with a lobular expansion continued by a slight depression near the articulation with the movable finger (Figs. 8, 9); with six carinae poorly developed, most of them barely visible as a slight elevation of the tegument with some setae, but the ventral carinae is well developed and bears two rows of setae, probably a fusion of two different carinae: DI and DS (in the basal half of the segment), DM, D, E and V + VM, extending the entire length of the segment. *Trichobothrial pattern*: neobothriotaxic major type C, with one accessory trichobothrium in the *V* series of chela; femur with 3 trichobothria (1 *d*, 1 *i* and 1 *e*); patella with 19 trichobothria (3 *V*, 2 *d*, 1 *i*, *3 et*, 1 *est*, 2 *em*, 2 *esb*, and 5 *eb*); chela with 27 trichobothria (1 *Est*, 5 *Et*, 5 *V*, 1 *Esb*, 3 *Eb*, 1 *Dt*, 1 *Db*, 1 *et*, 1 *est*, 1 *esb*, 1 *eb*, 1 *dt*, 1 *dst*, 1 *dsb*, 1 *db*, 1 *ib*, 1 *it*). *Hemispermatophore*: basal portion very developed; distal lamina short and curved, smaller than the basal portion; distal crest parallel to and almost transversal to the posterior margin, with a transversal crest; internal lobe with a small bilobate apophysis in its external surface that is not connected with the laminar apophysis (Fig. 2); lobe region well developed but very simple (Fig. 1), capsular concavity not very deep, restricted to the anterior margin of the basal lobe, but occupying most of the frontal surface of the lobe region; basal lobe very simple, without internal structures; internal lobe also very simple, almost completely covered by the basal lobe. We have dissected the hemispermatophores of seven specimens and have observed no conspicuous differences between them.

Distribution and habitat.—*Urophonius martinezi* has only been collected in Península Valdés and in an area very close to Puerto Madryn in central eastern Argentinean Patagonia (Fig. 21).

The Natural Protected Area Península Valdés (NPA-PV) is the largest unit of conservation of arid ecosystems of Argentina, it consists of a wide plateau of 4000 km^2 located in north-eastern Chubut province (42°05′–42°53′S, 63°35′–65°04′W) and has been declared Human Patrimony by UNESCO in 1999. Geologically it is formed by Oligo-Miocenic marine sediments and a continuous cover of aeolian sediments with quaternary gravels (Haller et al. 2001). Its actual landscape configuration was originated in the Pleistocene (~1 myrs) probably by strong periglacial winds that caused the deflation of the Gulfs of Nuevo and San José (Haller et al. 2001). The mean annual temperature is 14° C and the average annual precipitation is 175 mm in the coastal zone, with oscillations between 200 and 225 mm in internal zones (Barros & Rivero 1982). In this area, as in other arid ecosystems, the vegetation has a patchy structure alternating with bare ground (Bisigato & Bertiller 1997). The dominant physiognomy is a shrub steppe of *Chuquiraga avellanedae*, accompanied by *Chuquiraga hystrix* Don., *Condalia microphylla* Cav., *Lycium chilense* Miers, *Schinus polygamus* (Cav.), and *Prosopidastrum globosum* (Gill.). At the grass layer, the most common species are *S. tenuis*, *Piptochaetium napostaense* (Speg.) Hack., and *Poa ligularis* Nees (Bertiller et al. 1981). In the southern portion of NPA-PV the shrub steppe is replaced by an herbaceous steppe where *Sporobolus rigens* Desv. as the most important species, along with patches of *Ch. avellanedae* and *Hyalis argentea* D. Don (Bertiller et al. 1981; León et al. 1998). The NPA-PV has been the subject of several scientific contributions; however, there is conflict over its biogeographical identity. Soriano (1956) classified this area as belonging to the phytogeographical province of Patagonia. Cabrera & Willink (1973) included NPA-PV within the Monte province, whereas León et al. (1998) and Elissalde et al. (2002) described this region as an ecotone Monte-Patagonia. From a zoogeographical perspective its identity is still confusing because Morrone (2001a, 2001b) placed NPA-PV at the Patagonia Central province, while Roig-Juñent & Flores (2001) classified it as Monte region. The information about the epigean arthropod fauna from NPA-PV included in these contributions is mainly based on sporadic samplings and some references about the presence of a few isolated taxa (e.g. Cuezzo 1998; Flores 1998; Ceballos 2008; Crespo 2008; Ocampo 2008). Thus these contradictions about the biogeographical identity of NPA-PV are a consequence of a fragmentary knowledge about its epigeal arthropods fauna. The first ecological study in Península Valdés including an intensive seasonal sampling effort (from 2003 to 2006) is the one performed by the second author (Cheli, unpublished data). The presence of *Urophonius martinezi* in the Península Valdés, its closest relative being *U. eugenicus* (a typical southern Patagonian species), adds support to Morrone's (2001a) proposal of including NPA-PV in the Patagonian region, but future studies are needed to clarify this matter.

The actual distribution of *U. martinezi* cannot be accurately established with the scarce distribution data we have at this moment. With the information available, we can only conclude that this species occurs in the coastal area of central Chubut province. Some 200 km further north in Valcheta, southern Río Negro Province, it is replaced by *U. exochus*, and 400 km south in Caleta Olivia, in northern Santa Cruz province, it is replaced by *U. eugenicus* (Fig. 21). Our data show that *U. martinezi* has a restricted distribution (compared to other species of the genus), but we cannot be certain that it is restricted only to the NPA-PV and the area close to it, or if it occurs in a wider area of Chubut province.

Comments on the distribution and activity period of *Urophonius* in Patagonia.—In a previous contribution on the genus *Urophonius* (Ojanguren-Affilastro 2002) the first author has suggested that the Patagonian species of group *exochus*, *U. eugenicus*, and the species described in this contribution may be active on the surface during summer, whereas the rest of the species of the group are active at the surface during the winter. We based this conclusion on the dates of collection of the material deposited in different collections. However, the Patagonian specimens of *Urophonius* previously studied by us were old, manually collected material. The fact that these specimens were collected in the summer does not necessary imply that they were actually active in that period; most

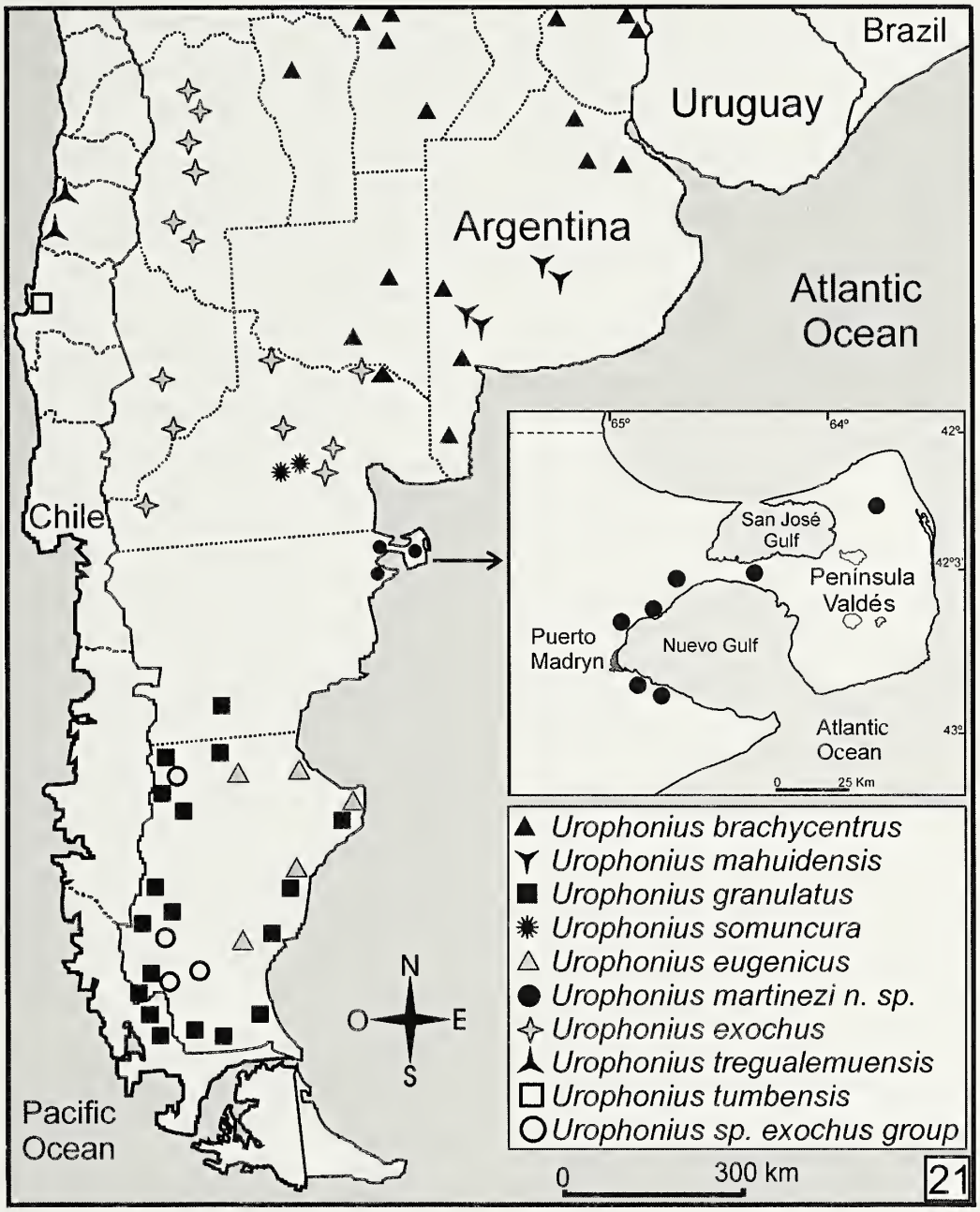


Figure 21.—Map of the southern part of South America, with the known distribution in this area of the species mentioned in this contribution. In detail, map of Península Valdés, with the known distribution of *Urophonius martinezi* new species.

probably this only reflects when most collecting trips in that area are carried out (during the summer), because of the extreme cold weather of the Patagonian winter. In a recent summer trip to Patagonia, no specimens of the *exochus* or *brachycentrus* group were observed during UV collecting at night (C.I. Mattoni pers. com.); the only active specimens of *Urophonius* observed at night were *Urophonius granulatus* Pocock 1898. In a recent trip to Patagonia in early winter (June–July 2008) we observed active specimens belonging to different species of *exochus* and *brachycentrus* groups: *U. exochus*, *U. eugenicus*, *U. martinezi*, and *Urophonius brachycentrus* (Thorell 1876), but no specimens of the *granulatus* group.

In addition, extensive collections from 2003 to 2006 performed in NPA-PV using pitfall traps by the second author have shown that the activity period of *Urophonius martinezi* is restricted to late autumn and winter (June, July, August), making it the only species of scorpion active in the area during this period of the year.

We have recently observed active populations of *Urophonius tregualemuensis* Cekalovic-Kuschevich 1981 in southern Chile during the spring and summer; this species of the *granulatus* group apparently has the same activity period as the Argentinean species of the *granulatus* group, *U. granulatus* and *Urophonius somuncura* Acosta 2003, which also have a spring-summer surface activity period (Maury 1979; Acosta 2003; Ojanguren-Affilastro 2005, 2007). The activity period of the other known species from southern Chile, *Urophonius tumbensis* Cekalovic-Kuschevich 1981, is still not known; we

could not collect, nor observe, any active specimen of this species in areas near the type locality during collection trips in the summer. The type material of this species could not be found in its depository at the MZUC (Raúl Briones Parra and Jaime Pizarro Araya pers. com.) and no other specimen has been collected since the original description of the species. Unfortunately the original description of *U. tumbensis* is a little vague, and we can not assign it to any of the groups of the genus. Our results show that all known species of *exochus* and *brachycentrus* groups have a winter surface activity period, whereas all known species of *granulatus* group have a spring-summer surface activity period. Taking into consideration that almost all bothriurid species show a spring-summer surface activity period, the winter activity pattern could be a synapomorphy for the *exochus* and *brachycentrus* group (C. Mattoni pers. com).

Recently we have collected specimens of *U. exochus* in a wide area of northern Patagonia (Neuquén and Río Negro provinces) belonging to the Monte phytogeographic province and to the ecotone between Monte and Patagonia phytogeographic provinces (Fig. 21). The known distribution of this species was restricted to Mendoza (in a slightly different environment) and to some probable records from Neuquén (Ojanguren-Affilastro 2005). These new records considerably expand the distribution of these species. We have observed slight morphological differences between specimens from different areas, but we prefer to consider them as intraspecific variation.

Acosta (1988) mentions the presence of a probable undescribed entity of the *exochus* group from Perito Moreno, in western Santa Cruz province, Argentina. We have examined a male specimen from this locality (probably the one studied by Acosta) and consider it to be an undescribed species. This species is closely related to *U. eugenicus*, but it has different morphometric proportions; is smaller and more densely pigmented. We have examined other specimens from southeastern Santa Cruz province, and they could also belong to this new species; however, they are poorly preserved, so we cannot assure this. Apparently this undescribed species inhabits areas close to the Andes mountain chain, whereas *U. eugenicus* occurs in the eastern part of the province.

New records for *Urophonius* species from Patagonia.—***Urophonius exochus*:** *Neuquén Province*: 6 ♂, 12 ♀, 18 juveniles, 30 km SW Zapala, (39°01′04.4″S, 70°09′21.2″W), 2 June 2008, Ojanguren-Affilastro, Compagnucci & Martinez (MACN-Ar); 7 ♂, 16 ♀, 23 juveniles, 20 km NE Piedra del Águila (39°58′46.5″S, 70°02′20.8″W), Ojanguren-Affilastro, Compagnucci & Martínez (MACN-Ar). *Río Negro Province*: 1 ♀, 1 juvenile, Pajalta, (40°45′0″S, 66°2′60″W), 18 August 1967, Maury (MACN-Ar); 1 juvenile, 60 km N Nahuel Niyeu, (40°30′04″S, 66°32′58.9″W), Bachmnn (MACN-Ar); 3 ♂, 4 ♀, 10 juveniles, 15 km N Valcheta, (40°43′05.7″S; 66°11′56.2″W), Ojanguren-Affilastro, Compagnucei & Martínez, 4 June 2008, (MACN-Ar); 1 ♂, 4 ♀, 8 juveniles, 8 km W. Choele-Choel, General Roca Monument (39°14′19″S, 65°40′48.7″W), Ojanguren-Affilastro, Compagnucci & Martinez, 3 June 2008, (MACN-Ar); 3 ♂, 5 ♀, 14 juveniles, 15 km S. Paso Córdova (General Roca), (39°07′29.7″S, 67°40′37.4″W), 31 May 2008, Ojanguren-Affilastro, Compagnucci & Martinez, (MACN-Ar); 1 ♀, Cerro Villegas, Estancia San Ramón, San Carlos de Bariloche, (41°04′10.86″S, 71°0.8′52.41″W), 17 July 1968, Muller, (MACN-Ar).

Comments.—Maury (1973) records the presence of *Urophonius mahuidensis* Maury 1973 in the locality of Paja Alta, in the base of the Somuncura plateau, based on a female and a juvenile specimen; Ojanguren-Affilastro (2002, 2005) repeats this. Unfortunately the identification of the specimens of this group is very difficult and in some cases it is only possible to identify the male specimens. We have been able to study more material from that area (including males) and we conclude that the specimens studied by Maury actually belong to *U. exochus*, or at least to a species very closely related to it, but not to *U. mahuidensis*. According to our results *U. mahuidensis* is endemic to the Tandilia and Ventania mountain chains in southern Buenos Aires province, Argentina, the same as *Botriurus voyatti* Maury 1973 (Maury 1973).

***Urophonius eugenicus*:** *Santa Cruz Province*: 8 ♀, 16 juveniles, 15 km S Caleta Olivia, near Cañadón Seco (46°30′36.6″S, 67°27′48″W), 7 June 2008, Ojanguren-Affilastro & Martinez, (MACN-Ar); 5 ♀, 7 juveniles, 20 km W. Las Heras (46°33′35″S, 69°02′23.2″W), 8 June 2008, Ojanguren-Affilastro & Martinez, (MACN-Ar); 17 ♂, 5 ♀, 20 juveniles, 8 km N. Puerto Deseado, (47°42′42,43″S, 65°50′15.68″W), 6 June 2008, Ojanguren-Affilastro & Martinez, (MACN-Ar).

Comments.—We have collected this species in the Eastern part of Santa Cruz province. Specimens from Western Santa Cruz, previously mentioned as belonging to *U. eugenicus* (Ojanguren-Affilastro 2002, 2005) belong to an undescribed species.

***Urophonius brachycentrus*:** *Río Negro Province*: 1 ♂, 3 ♀, 8 juveniles, 30 km E Choele-Choel, (39°15′27.7″S, 65°35′59.3″W), 3 June 2008, Ojanguren-Affilastro, Compagnucci & Martinez, (MACN-Ar).

Comments.—This is the first time this species has been collected in Río Negro province; however, it was previously collected in nearby areas from the surrounding provinces of Buenos Aires and La Pampa. In the locality of Cholel-Choel, this species has been collected very close to *U. exochus*; however, both species have different habitat preferences; *U. exochus* apparently prefers slopes and areas with some rocks, whereas *U. brachycentrus* prefers plains. In the nearby locality of Paso Cordova, where *U. brachycentrus* is not present, *U. exochus* is present in both types of environment, but it is more abundant on slopes than in plains.

KEY TO THE PATAGONIC SPECIES OF *UROPHONIUS*

1. Ventral surface of metasoma with two VL and a VM stripe (Fig. 19) ..... *granulatus* group 2
   Ventral surface of metasoma with two VL and two VSM stripes (Fig. 20) 4
2. *e* trichobothria of pedipalp femur placed proximally with respect to M1 macroseta (Fig. 15) ..... *Urophonius granulatus*
   e trichobothria of pedipalp femur placed distally or in the same axis with respect to M1 macroseta (Fig. 15) 3

3. *e* trichobothria of pedipalp femur placed on the same axis or slightly distally with respect to M1 macroseta (Fig. 15) ..... *Urophonius sonuncura*
   e trichobothria of pedipalp femur placed clearly more distally than M1 macroseta (Fig. 15) ..... *Urophonius tregualemuensis*
4. Pedipalp femur with two macrosetae (M1 and M2) associated with *d* and *e* trichobothria (Fig. 16) ..... *brachycentrus* group ..... *Urophonius brachycentrus*
   Pedipalp femur with one macroseta (M1) associated with *d* and *e* trichobothria (as in *granulatus* group) (Fig. 15) .... *exochus* group 5
5. Bilobate protuberance of the hemispermatophore connected to the distal lamina (Fig. 5) ..... *Urophonius mahuidensis*
   Bilobate protuberance of the hemispermatophore not connected to the distal lamina (Figs. 2, 3, 4) 6
6. Bilobate apophysis of the hemispermatophore very close to the distal lamina, but not forming a part of it; distal lamina slender, almost straight, anterior margin slightly curved (Fig. 4) ..... *Urophonius exochus*
   Bilobate apophysis of the hemispermatophore clearly separated from the distal lamina; distal lamina stout, anterior margin strongly curved (Figs. 2, 3) 7
7. Vesicle of the telson globose, highly developed toward the anterior margin (Figs. 12, 14), length/height ratio of telson in males: 2.54–3.03, $n = 18$, median = 2.85. Pigment pattern of prosoma occupying most of the area between the ocular tubercle and the lateral eyes (Fig. 17) ..... *Urophonius eugenicus*
   Vesicle of the telson slender, especially in males, in females it is globose but not highly developed towards the anterior margin (Figs. 11, 13), length/height ratio of telson in males: 3.10–3.52, $n = 20$, mean = 3.28. Pigment pattern of prosoma poorly developed, area between the ocular tubercle and the lateral eyes almost unpigmented (Fig. 18) ..... *Urophonius martinezi*

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Foraging strategies and diet composition of two orb web spiders in rice ecosystems

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Abstract. We conducted a field study in September 2007 and 2008 to analyze the foraging activity, natural diets, and predatory efficacy of *Tetragnatha javana* (Thorell 1890) (Araneae: Tetragnathidae) and *Neoscona theis* (Walckenaer 1842) (Araneae: Araneidae) on selected prey. The relationship between body measurements (carapace width, leg length, total body length, and body weight) and web dimensions (capture area, capture thread length, number of radii, number of spirals, and mesh height) of both species was also investigated. Most of the observed *T. javana* constructed their webs between two adjacent rice plants, while *N. theis* placed theirs at the top of riee plants. Both species required approximately an hour to complete a web, which differed significantly from each other in height, diameter, and capture area. Both species constructed only a single web per day. Web building activity of both species was intense from 17:00 to 18:00, while prey-handling activity was high from 19:00 to 20:00. In both species, peaks of feeding were recorded just after the peaks of prey handling (21:00). The main prey orders caught in the webs of both species were Lepidoptera, Diptera, Homoptera, Coleoptera, Hymenoptera, and Orthoptera. The time required to reach and capture lepidopteran (adults of stem borer and leaf folder) and homopteran prey was similar for both species. However, the time required to reach and eapture orthopteran (grasshopper nymphs) prey was significantly longer for *T. javana* than for *N. theis*. Capture area increased with carapace width, and capture thread length increased with carapace width and body weight, while leg length and body length did not relate to either of these web variables. The number of radii, number of spirals, and mesh height did not correlate with any of the body size measurements. We concluded that both species can be used effectively to reduce insect pests of rice fields.

Keywords: Araneidae, agroecosystem, pest suppression, biological control

Spiders are among the most abundant predatory groups in rice ecosystems (Sebastian et al. 2005; Takashi et al. 2006; Tahir & Butt 2008). Most of them are polyphagous predators, able to feed on various insect pests of agricultural crops (Lang et al. 1999; Hanna et al. 2003; Schmidt et al. 2004; Takashi et al. 2006). Several studies clearly describe their role in reducing insect pests in rice fields (Xu et al. 1987; Ye & Wang 1987; Tanaka 1989; Jalaluddin et al. 2000). Spiders use a variety of methods to capture prey. Hunting spiders may actively pursue or ambush prey, while web building spiders present a unique case of "sit-and-wait" predation (Heiling 1999; Park et al. 1999). Orb web spiders are characterized by the use of a web to capture prey (Turnbull 1960; Kajak 1965). Prey capture success of web building spiders is also influenced by spider size (Eberhard 1990), web size (Sherman 1994), and web placement (Chacon & Eberhard 1980; Rypstra 1985) as well as specific web parameters such as strength (Lubin 1986), adhesiveness (Opell 1994), extensibility (Vollrath 1992), and mesh size (Rypstra 1982; Eberhard 1986).

Orb web spiders may trap more insects than they can consume. Silk of some orb web weavers attracts herbivorous insects that would normally be drawn to flowers and new leaves (Craig et al. 1996). Up to 1000 insects may be present in a web at a given moment, and many are left in the web to be eaten later (Nyffeler et al. 1994a). Small pests, such as thrips, midges, and aphids, may be caught and die in the webs of large spiders, only to be ignored by the spiders (Nentwig 1987; Landis et al. 2000). Web weaving spiders must anchor their prey-capture devices to the appropriate substratum in order to increase the effectiveness of their webs; complex habitats provide appropriate sites for different sizes and types of webs in prey capture (Rypstra et al. 1999).

Orb webs have developed as an efficient means of capturing flying insects. The optical properties of these webs tend to reduce their visibility, especially in low-light and varying background conditions (Craig 1986), making detection and avoidance difficult (Robinson & Mirick 1971). There are numerous reports concerning prey captured by web building spiders (Heiling 1999; Ibarra-Nunez et al. 2001; Ceballos et al. 2005).

We designed the present study to understand the role of two nocturnal orb web spiders, *Tetragnatha javana* (Thorell 1890) and *Neoscona theis* (Walckenaer 1842), in the suppression of insect pests in rice fields. These species were selected because of their abundance in the rice ecosystems of central Punjab, Pakistan (Tahir & Butt 2008). The objectives of the study were to record the differences, if any, in web building, prey handling, and feeding activities of both orb web weavers, to study the relationship of body size measures (carapace width, leg length, total body length, and body weight) with various web characteristics (capture area, capture thread length, number of radii, number of spirals, and mesh height), and to record the difference in time elapsed to reach and capture prey blown experimentally into their webs. This study will help to understand the impact of these orb web spiders in the suppression of insect pests of rice.

METHODS

Study site.—The study was conducted in September 2007 and 2008 in rice fields at the agricultural research farm,

Sheikupura (31°43′N, 73°59′E). The rice variety grown was super basmati. At the time of the experiment, the average height of the plants in the fields was 131 ± 11 cm). During the course of the study, the temperature fell to approximately 27 ± 4° C at night, and rose to about 41 ± 6° C during the day. The relative humidity was highly variable (65–85%).

Field observations.—We identified *T. javana* and *N. theis* by consulting Barrion & Litsinger (1995). We conducted field observations on three different days in the third and fourth week of September (2007 and 2008), respectively, starting each day at 16:00 h. During the study period, sunset occurred between 18:00 and 18:30 h and sunrise between 05:00 and 06:00 h. To check the activities of spiders and their webs, we walked through the field every hour during 24 h to cover a 50 × 50 m plot (the walk required 20 to 25 min). At night, we used a flashlight covered with dark red plastic, because it neither attracted insect prey nor disturbed the spiders' natural photoperiod (Herberstein & Elgar 1994; Heiling 1999; Ceballos et al. 2005). On each walk we recorded adult female spiders present on rice plants with or without a web. Each spider's position was marked individually with a numbered piece of white plastic tied to the nearest twig. Spider activities recorded were resting, building a web, handling or eating a prey. Prey counted included both those fed upon at the hub and ones tangled in the web but not being fed upon. Prey items in the webs of spiders were identified to order.

Information regarding web diameter, web position (height from the ground and location on the rice plant), capture area, capture thread length, mesh size, number of radii, number of spirals, and time required to complete a web were also collected (n = 50 each year). To record the data, we removed spiders from the webs and sprayed their webs with a fine mist of water and cornstarch, using a knapsack hand sprayer (THS-119428) to improve the resolution. Similarly, carapace width, length of leg IV (coxa to tarsus), total length, and weight were also recorded. Since the data did not differ during the two years (for either species), they were pooled for statistical analysis.

To estimate the number of prey items per m^2 of rice plants, we quickly covered plants with two plastic bags, and then cut all plants just above the roots. Arthropod prey were sampled every two hours (three replicates). Each entire cut rice stem was brought to the laboratory and carefully examined for insects. Total webs per m^2 were also counted every two hours (three replicates).

The normality distribution of the data was analyzed with Kolmogorov-Smirnov tests before conducting further statistical analysis. Student t-tests were applied to normally distributed data. Relationships between body size (carapace width, leg length, body length, and weight) and web size (capture area, capture thread length, mesh size, number of radii and number of spirals) were analyzed using Pearson correlations (Minitab 13.3). Data are presented as mean ± 1 SD.

Prey capture efficiency.—We conducted an experiment to record prey capture events in the field during the third week of September 2007. In order to record the prey-capture efficiency of web weaving spiders, we used four experimental prey types that are major rice insect pests in the study area: adults of whitebacked planthoppers *Sogatella furcifera* (Horvath), leaf rollers *Cnaphalocrocis medinalis* (Guenee), white stem borers *Scripophaga innotata* (Walker), and nymphs of grasshoppers *Hieroglyphus banian* (Fabricius). Twenty-five individuals of each prey type (plant hoppers: 3.4 ± 0.6 mm, grasshoppers: 10 ± 2.3 mm, stem borers 9 mm ± 2.4 mm, leaf folders 10 ± 3.4 mm) were collected with a sweep net (42 × 80 cm, with a 90 cm handle) and a suction device (SIEMENS VK 20C01). Webs of adult females with no signs of prey were used in the experiment (Sebastian et al. 2005). Each prey animal was gently blown into the web with an inverted aspirator from 10 cm away. An average of twenty replicates was used for each type of prey, using a different web for each item. All prey were alive and undamaged before and after their introduction into the web. Once the prey contacted the web, we measured how long the spider needed to approach and capture the prey. Prey handling time began when the spider bit the prey, continued as the prey was manipulated, and ended when the spider took the prey to the hub. The predatory efficiency of both web-weaving spiders on four experimental prey types was compared using two-way ANOVA (SPSS 13). Subsequently, we conducted a Tukey HSD test separately for the time it took the spider to reach the prey and to capture it, with one factor being the spider species (2 levels) and the other being the prey species (4 levels).

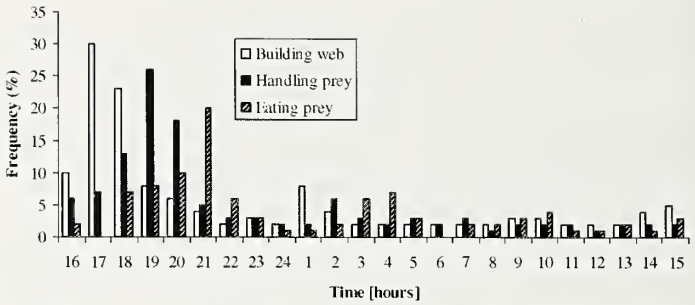

Figure 1.—Relative frequency of web building, prey handling, and feeding individuals of *Tetragnatha javana* each hour during 24 h in rice fields.

RESULTS

Tetragnatha javana.—A total of 214 spiders (123 in 2007 and 91 in 2008) and 135 webs (77 in 2007 and 58 in 2008) was observed during experimental periods of three days and nights. Although *T. javana* built webs at all hours of the day and night, their web building activity was most intense between 16:00 and 20:00 h (Fig. 1). Of the total observed, 59% completed web building between 17:00 and 18:00 h. *T. javana* constructed their webs between two adjacent rice plants and required 1 ± 0.3 h to complete a web. Webs averaged 109 ± 7.3 cm high and 29 ± 3.9 cm wide, n = 50 both years). After constructing a web *T. javana* occupied the center of the web and quickly attacked prey that attempted to escape.

Prey handling of *T. javana* was most intense between 19:00 and 20:00 h and decreased until 02:00 h, just after a second peak of web building. Feeding activity of *T. javana* was highest at 21:00 h, with a smaller peak between 03:00 and 04:00 h (Fig. 1). Combining both years, we observed 135 webs that contained 993 prey items (mean = 7.4 prey/web: Lepidoptera (41%), Diptera (24%), Homoptera (15%), Coleoptera (6%), Hymenoptera (3%), Orthoptera (3%), Araneae (3%) and unidentified prey (5%) (Table 1).

Table 1.—Relative frequency of pests recorded from the webs of *Tetragnatha javana* (n = 993) and *Neoscona theis* (n = 849).

Common name	Scientific name	*T. javana*	*N. theis*
Lepidoptera			
Yellow stem borer	*Scripophaga incertulas* (Walker)	14	11
White stem borer	*Scripophaga innota* (Walker)	12	13
Pink stem borer	*Sesamia inferens* (Walker)	4	3
Stripped borer	*Chilo suppressalis* (Walker)	3	2
Sorghum stem borer	*Chilo partellus* (Swinhoe)	1	–
Leaf folder	*Cnaphalocrocis medinalis* (Guenee)	5	4
Leaf folder	*Mythimna separata* (Walker)	2	3
Homoptera			
Whitebacked planthopper	*Sogatella furcifera* (Horvath)	7	9
Green leafhopper	*Nephotettix nigripictus* (Stal.)	4	4
White leafhopper	*Cofana spectra* (Distant)	4	3
Diptera			
Rice gall midge	*Pachydiplosis oryzae* (W.-M.)	14	14
Rice shoot fly	*Atherigona oryzae* (Mall.)	6	4
Rice shoot fly	*Atherigona soccata* (Rond.)	3	3
Mosquito	*Culex* spp.	1	–

Density of potential prey increased after 16:00, reached a maximum at 20:00, and then declined until 04:00. Frequency of prey handling increased with density of potential prey (r = 0.63; $P < 0.01$, Fig. 1).

Neoscona theis.—We observed 141 spiders (73 in 2007 and 68 in 2008) and 97 webs (57 in 2007 and 40 in 2008). Of these, 63% completed web building between 17:00 and 18:00 h. Web building activities decreased but did not cease throughout the night (Fig. 2). Most *N. theis* (67%) constructed their webs at the top of rice plants and required 1 ± 0.4 h to build their webs. The hub of the web averaged 128 ± 7.0 cm above the ground, and the average diameter of the web was 34 ± 4.7 cm (n = 50 both years).

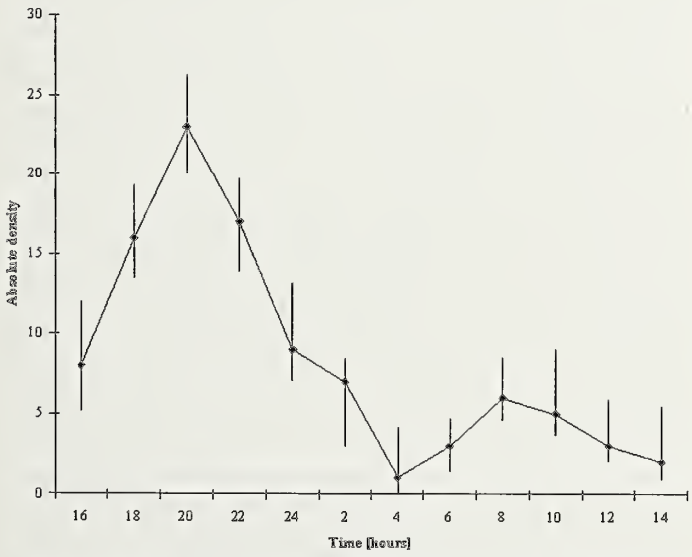

Figure 2.—Mean density (± SD) of potential prey per 1 m^2 of rice fields recorded at each two-hour period during 24 h (combined for both years).

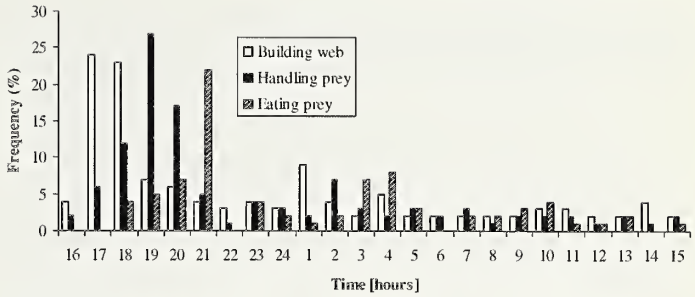

Figure 3.—Relative frequency of web building, prey handling, and feeding individuals of *Neoscona theis* each hour during 24 h in rice fields.

Prey handling activity of *N. theis* was most intense between 19:00 and 20:00 h, and increased slightly again around 02:00 h, just after the second highest frequency of web building. Feeding peaked at 21:00 h, 1 h after peak prey handling activity. A second minor peak in feeding occurred between 03:00 and 04:00 h. Web building, prey handling, and feeding activities of *N. theis* decreased before 06:30 (Fig. 3).

A total of 849 insects was recorded from 97 webs, an average number of 8.8 prey/web over the two years combined, consisting of Lepidoptera (36%), Diptera (21%), Homoptera (16%), Coleoptera (8%), Orthoptera (8%) Hymenoptera (4%), Araneae (4%), and unidentified prey (3%) (Table 1). Frequency of prey handling increased with density of potential prey (r = 0.59; $P < 0.01$, Fig. 3).

Predatory efficacy.—The time required for the two spiders to reach prey differed (two-way ANOVA: $F_{1,\ 23} = 29.54$, $P < 0.01$, Table 2). Time to reach prey also differed among four prey types ($F_{3,\ 23} = 359.93$, $P < 0.001$). Similarly, the time to capture prey differed between the two species ($F_{1,\ 23} = 209.33$, $P < 0.001$), as well as among the four prey types ($F_{3,\ 23} = 296.41$, $P < 0.001$, Table 3). Differences were due to the handling of orthopteran prey (Tukey HSD test).

Relationship of body size and web design.—The web design of adult females of both species correlated differently with the various body size measurements (Table 4). Capture areas of both species' webs increased significantly with carapace width (r = 0.51, $P < 0.01$ for *T. javana*; r = 0.54, $P < 0.01$ for *N. theis*), and capture thread length increased significantly with carapace width (r = 0.55, $P < 0.01$ for *T. javana*; r = 0.62, $P < 0.01$ for *N. theis*), and body weight (r = 0.60, $P < 0.01$ for *T. javana*; r = 0.68, $P < 0.01$ for *N. theis*). Leg length and body length did not correlate with these two web variables. Neither did the number of radii, number of spirals, and mesh height correlate with any of the four measurements of body size ($P > 0.05$). Comparison of web height, diameter, and capture area of the two species differed significantly (t_{48} = 4.24, $P < 0.01$; t_{48} = 3.87, $P < 0.01$; t_{48} = 11.9, $P < 0.001$, respectively). However, the number of spirals and number of radii in the webs of these two orb web spiders did not differ significantly (t_{48} = 0.42, $P > 0.05$; t_{48} = 0.72, $P > 0.05$, respectively).

DISCUSSION

This study suggests that most individuals of both species started to build their webs just after sunset, and kept the activities of web building, prey handling, and prey eating to a minimum after sunrise. Most individuals of both species fed

Table 2.—Mean time (s, ± SE) to reach four prey types by *Tetragnatha javana* and *Neoscona theis*. Row-wise comparisons were done by Tukey HSD test. * $P < 0.005$; ns = non significant.

Prey type	*T. javana*	*N. theis*	Comparison
Scripophaga innotata (Walker)	9.5 ± 1.0	12.1 ± 1.3	ns
Cnaphalocrocis medinalis (Guenee)	8.1 ± 2.1	11.7 ± 3.1	ns
Sogatella furcifera (Horvath)	9.1 ± 2.2	11.4 ± 2.0	ns
Hieroglyphus banian (Fabricius)	31 ± 1.4	14.2 ± 3.5	*

throughout the observation period; they seem to have a strategy to "build, catch, and eat" in a short period (Ceballos et al. 2005). Prey handling rates were highest at the beginning of the night in both species due to the high level of prey activity at this time (Kraker et al. 1999). Prey handling decreased after 20:00, which might be due to decreased activity of the prey after 20:00.

Both spiders took less time to reach and capture adult stem borers, leaf folders, and planthoppers than grasshopper nymphs in the prey capture efficacy experiment. This difference might be due to lower efficiency of their venom. Small prey were usually paralyzed more quickly than larger ones.

Although the main prey items of both web weavers were Lepidoptera, they both also fed on Diptera, Homoptera, Hymenoptera, Coleoptera, and Orthoptera. A comparable composition (insect orders) of potential prey for the web building spiders *Araneus diadematus* Clerck 1757 and *Argiope bruennichi* (Scopoli 1772) was described by Ludy (2007). These insect orders make up the majority of prey of spiders in rice agroecosystems. A varied diet creates an optimal, balanced nutrient composition needed for survival and reproduction (Greenstone 1979; Toft 1995). However, prey groups were not caught in the spider webs in proportions to their availability in the habitat. For example, we recorded 180 plant hoppers from 1 m^2 of experimental rice field during a high abundance period in the last week of September, but only 21 (11.7%) from the webs in this area.

Members of insect orders with good vision and maneuverability in flight, such as Diptera and Hymenoptera (Land 1997), may detect and evade webs, resulting in an underrepresentation there. However, good vision and maneuverability in flight is not important in the present study because both of the species captured prey mainly during night when visibility was low. Small and slow-flying insects with relatively large surface areas may be caught in spider webs most easily

Table 3.—Mean time (s, ± SE) to capture four prey types by *Tetragnatha javana* and *Neoscona theis*. The prey capture started from the first contact with the prey and ended when the spider took the prey to the hub. Row-wise comparisons were done by Tukey HSD test. * $P < 0.005$; ns = non significant.

Prey type	*T. javana*	*N. theis*	Comparison
Scripophaga innotata (Walker)	19.2 ± 3.0	24.4 ± 1.7	ns
Cnaphalocrocis medinalis (Guenee)	23.4 ± 2.6	27.0 ± 0.0	ns
Sogatella furcifera (Horvath)	19.7 ± 2.1	17.0 ± 4.3	ns
Hieroglyphus banian (Fabricius)	84.2 ± 12.8	42.4 ± 5.1	*

Table 4.—Summary of web characteristics and body measurements (mean ± SE) of adult females of *Tetragnatha javana* and *Neoscona theis*.

Characteristic	*T. javana*	*N. theis*
Web height (cm)	109.7 ± 7.3	128.0 ± 7.0
Web diameter (cm)	29.3 ± 3.9	34.0 ± 5.0
Capture area (cm^2)	91.1 ± 9.4	126.7 ± 16.3
Number of radii	15.0 ± 3.0	17.0 ± 4.0
Number of spirals	22.0 ± 4.0	27.0 ± 4.0
Mesh height (mm)	1.6 ± 0.6	2.7 ± 0.3
Carapace width (mm)	1.0 ± 0.3	1.9 ± 0.4
Leg IV length (mm)	12.0 ± 1.4	11.0 ± 1.5
Total length (mm)	11.5 ± 0.5	7.4 ± 1.3
Wet weight (mg)	13.0 ± 7.34	110.7 ± 39.0

(Kajak 1965; Nentwig 1982, 1985). In the present study, more than 70% of the prey caught in the webs of both spiders belonged to the Lepidoptera, Diptera and Homoptera. More than 90% of the prey items recorded from the webs of both species were insects - the remaining 10% were spiders and unidentified prey. The difference in abundance of prey at different times is due to the difference in activity of insects at those times.

The webs of orb weaving spiders vary greatly in design; scientists have interpreted this variation as specialization for the capture of specific prey types (Walker 1992). Much of the interspecific variation in web architecture is related to factors other than prey types, including amount or shape of available space, presence of conspecifics, lack of previous experience at a website, presence or absence of water immediately below the orb, amount of silk available in the glands, and time of day (Eberhard 1990). Webs of smaller spiders, which are generally made with thinner threads and less adhesive, have reduced abilities to capture large prey (Eberhard 1990). Variation in web design (position, height, capture area, number of radii, hub position, number of spirals) of both species, as well as variation within species, was also recorded in this study. The general web architecture is thought to be genetically determined (Foelix 1992). Capture area and capture thread length increased significantly with carapace width in this study, a result also reported by Heiling et al. (1998). A large capture area results in high prey interception (Chacon & Eberhard 1980), and by increasing the distance between sticky spirals, spiders may enlarge the overall capture area without increasing their energy expenditure (Herberstein et al. 2000). Our result is in accordance with previous studies that also found a positive relationship between carapace width and web size (Eberhard 1988; Heiling et al. 1998). Mesh height did not relate to any of the body measurements in the present study, contrasting with the results of Eberhard (1988), who found leg length to be a good indicator of mesh height. Numerous field studies have also failed to find a consistent relationship between mesh height and prey size (Herberstein & Elgar 1994; Herberstein & Heiling 1998). A narrow mesh may facilitate the retention of larger prey, as more threads are in contact with the item (Eberhard 1990). However, more spiral turns also reflect more light, thus increasing the visibility of the web to the prey (Craig 1986; Craig & Freeman 1991). Mesh height may therefore indicate a compromise between prey retention and web visibility.

The possible role of these spiders in pest control can be estimated with simple calculations. The average number of pests and webs from 1 m^2 of rice fields of Punjab were 140 and 3.5, respectively. The average number of pests recorded from a single web of *T. javana* was 7.4, while the average number of pests collected from a single web of *N. theis* was 8.8. Thus, these two web builders (*T. javana* and *N. theis*) can produce up to a 22% reduction of the total pest population per day. Furthermore, many other spider species and other natural predators also contribute to the suppression of insect pests in the rice ecosystem. Both spiders studied can reduce the populations of insect pests in rice fields and may be useful in biological control of rice insect pests in Pakistan. However, in order to use them as biological control agents on a broader scale, further knowledge of their feeding habits, web construction behavior, reproductive strategies, prey preferences, and response to insecticides and herbicides is needed.

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BOOK REVIEW

Dominican Amber Spiders: a Comparative Palaeontological-Neontological Approach to Identification, Faunistics, Ecology and Biogeography. By David Penney. 2008. Siri Scientific Press, Manchester, UK. 176 pp. ISBN 978-0-9558636-0-8. £40.

David Penney is a Visiting Research Fellow at the University of Manchester, UK and an expert on fossil spiders preserved in amber. This beautifully illustrated book has in excess of 330 figures, including more than 80 high-quality color photos. The author uses a unique, integrated approach, that combines and compares information derived from both fossil and living spiders. There are eight main sections, the first being a general introduction to amber, classification of spiders and their fossil record, as well as information on other important fossil localities, modes of preservation, the timing of important radiations in spider evolutionary history, co-evolution with their insect prey and the effects of mass extinction events. Much of this information is neatly illustrated in an accurate and comprehensive figure of the spider evolutionary tree.

Chapter 2 provides thorough coverage of the current state of knowledge with regard to Dominican amber, including geological age and botanical origin, with a fully referenced discussion of competing ideas. This is followed by sections on chemical and physical properties, how to identify fakes, and a comprehensive coverage of tissue preservation in amber, including whether or not it is possible to extract DNA. A full description of the journey of amber from mine to museum is presented, based on the author's own experience of visits to the amber mines. This is followed by an extremely useful section on the methods of amber preparation and study, including general and advanced photography and microscopy, with coverage and amazing images of new techniques such as those provided by x-ray computed tomography. In particular, this section is broadly applicable to any amber and brings together information from a great deal of literature, which is often hidden in obscure specialist journals. This chapter ends with short discussions on the major world collections of Dominican amber inclusions, how to conserve and curate amber collections and the current state of knowledge of biodiversity of all fossils preserved in Dominican amber. This is fully up-to-date, and readers are directed to the most important publications on Dominican amber biodiversity, including the most recent, which lists all 1,404 fossil species known to occur in Dominican amber.

Chapters 3 to 5 are more spider orientated, the first providing an interesting historical account of Hispaniolan spider research with regard to both the extant and fossil faunas, which are treated separately. All publications describing new species or providing new records for the island are included. This is followed by a full checklist by family, including both fossil and extant species (495 species in 52 families), with the fossil species clearly indicated. It is worth noting at this point that no new species are described in this book, but one new combination is proposed. Chapter 4 is a fully illustrated key to Hispaniolan spider families, with a strong emphasis on those characters that are likely to be observed in fossils. The key is somewhat limited in scope because it refers only to Hispaniolan species (although that is the purpose of the book). For example, the author distinguishes Nephilidae from Tetragnathidae by the presence of humps on the carapace of the former. Whilst this holds true for the Neotropical *N. clavipes* it is not the case for all species in this genus. In my opinion, there are a few other incorrect statements within the key, but it is impossible to avoid such inaccuracies in rather generalized dichotomous keys. The key is preceded by suites of highly distinctive characters with regard to general body morphology, which will negate the need to use the key in many instances and allow the reader to skip directly to the next chapter, which consists of the family descriptions. Each family entry contains the following subheadings: Dominican amber, extinct taxa; Hispaniola, extant taxa; Identification; Natural history; Relevant publications; Additional notes. Again, this chapter is beautifully illustrated throughout with photographs and illustrations of both fossil and living spiders. Whilst the general aim is to permit identification only to family level, in many cases it will allow identification to genus and species. The correct assignment of some species attributed to various families by earlier workers is questionable in my opinion, but in most instances this is not discussed, presumably to maintain a balance for non-arachnologists who are interested in amber spiders.

Chapter 6 covers various aspects of palaeoecology and historical biogeography, with particular reference to spiders, but is of much broader significance with regard to the fossil amber fauna and Caribbean biogeography in general. Topics covered include the site of resin secretion, the entrapment process, whether different ambers trap organisms in the same way, bias in the amber fauna, comparison of fossil and extant faunas. All information is easily accessible and graphically portrayed, using excellent color figures. An extensive analysis of the biogeographic origins of Hispaniolan spiders is published here for the first time, based on large datasets of spider distribution and the current knowledge of the geological origins of the Caribbean. Once again, the scope of this chapter extends far beyond the amber spider world. This chapter ends with predictions that can be made for the Hispaniolan spider fauna based upon what is known about the fossils. Chapter 7 provides a useful referenced checklist of all

other arachnids described from Dominican amber, including 18 families of Acari, in addition to the orders Amblypygi, Opiliones, Pseudoscorpiones, Scorpiones and Solifugae. The book ends with an extensive bibliography of more than 350 entries, providing an invaluable resource for anybody interested in amber or Caribbean spiders, followed by an index. Unfortunately, the index does not list spiders by genera, although families are listed and it is not too much effort to determine whether or not a genus is included from the Hispaniolan spider checklist in Chapter 3 or from the relevant family page in Chapter 5. The page number for *Megarachne* has also been accidentally omitted. In addition there are several typos in the text and very minor inconsistencies. For example, the orientation of the spider evolutionary tree relative to the page differs on pages 18 and 138.

In summary, this book will be of considerable interest beyond the Dominican amber spider world and represents a very important contribution to studies on Caribbean biogeography and palaeobiogeography, the literature on amber, the fauna of Hispaniola (both fossil and extant), and an identification aid for workers in the Caribbean region. David Penney is undoubtedly the world expert in this field and has compiled a comprehensive synthesis with beautiful illustrations on almost every page, making it a pleasure to the eye. The information is sound and reliable, the bibliography extensive and complete, and the text is authoritative. There is no other work available quite like it. Despite the minor shortcomings mentioned above, I evaluate this book very highly and would recommend it to be on the bookshelves of academic libraries and museums, in addition to those of people with a general interest in spiders or amber, both amateur and professional.

The book is available from the publisher (http://www.siriscientificpress.co.uk, email:) or from the author via e-mail (david.penney@manchester.ac.uk).

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SHORT COMMUNICATION

Habitat selection and potential antiherbivore effects of *Peucetia flava* (Oxyopidae) on *Solanum thomasiifolium* (Solanaceae)

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Abstract. Several spider species use plants as shelter and foraging sites, but the relationships among these organisms are still poorly known. Lynx spiders of the genus *Peucetia* do not build webs, and many species live strictly in plants bearing glandular trichomes. *Peucetia flava* Keyserling 1877 inhabits *Solanum thomasiifolium* in southeastern Brazil and usually preys on herbivores and other small insects adhered to the glandular trichomes of its host plant. To evaluate the potential anti-herbivore protection of this spider species for *S. thomasiifolium*, we glued termites used as herbivore models on trichomes of *S. thomasiifolium* and on neighboring plants lacking glandular trichomes. Leaf miner damage and spider density were recorded for *S. thomasiifolium* plants in July 1997. There was a positive relationship between plant size and spider density. The removal of termites in *S. thomasiifolium* by *P. flava* was higher than in plants without glandular trichomes. The leaf miner damage was negatively related to spider density. Our results suggest that *P. flava* may be an important plant bodyguard in the defense of *S. thomasiifolium* from its natural herbivores.

Keywords: Animal-plant interactions, host plant specificity, lynx spider, plant protection

It is widely known that several spider species use plants as shelter and foraging sites (Foelix 1996), but only in the last few decades have strict associations of spiders with particular plant types or species been described in detail. A variety of spider-plant associations has been described in the Neotropical regions (Barth et al. 1988; Dias & Brescovit 2004; Romero 2006). The associations typically occur because plants have morphological traits that provide suitable foraging, mating, and egg-laying sites for the spiders, shelter for adults and immatures, and nurseries for spiderlings (Romero & Vasconcellos-Neto 2005), thereby improving the probability of the spiders living on them. One of these associations includes jumping spiders (Salticidae) that are strictly associated with Bromeliaceae (Romero & Vasconcellos-Neto 2004a, 2004b, 2005; Romero 2006). For one of these associations, Romero et al. (2006) demonstrated through experiment that the jumping spider *Psecas chapoda* Peckham & Peckham 1894 contributed to 18% of the total nitrogen of its host terrestrial bromeliad *Bromelia balansae*, and in general, plants with spiders produced leaves 15% longer than plants from which the spiders were excluded.

Other associations involve spiders that forage on plants with glandular trichomes. For instance, at least 10 lynx spider species of the genus *Peucetia* (Oxyopidae) live strictly in several plant families and species bearing glandular trichomes in many distinct vegetation types in Neotropical, Nearctic, Palearctic, and Afrotropical regions. The main plant families used by these spiders are Solanaceae, Asteraceae, and Melastomataceae (Vasconcellos-Neto et al. 2007). The specialization of the *Peucetia* species for plants bearing glandular trichomes may have evolved because insects adhering to these sticky structures may be used as prey by the spiders (Vasconcellos-Neto et al. 2007). Although glandular hairs have probably evolved as a defense against herbivores and pathogenic fungi (Levin 1973, Duffey 1986), they can also mediate mutualistic interactions between plants and spiders or other predators (Dolling & Palmer 1991; Romero et al. 2008).

In South America, *Peucetia rubrolineata* Keyserling 1877 and *P. flava* Keyserling 1877 are the most common representatives of the genus. Both species are widely distributed throughout Brazil and occur sympatrically in several localities (Santos & Brescovit 2003; Vasconcellos-Neto et al. 2007). In a forest reserve in the state of Espírito Santo, southeastern Brazil, *P. flava* is frequently observed in association with *Solanum thomasiifolium* Sendtn. 1846, a solanaceous plant bearing glandular hairs (Vasconcellos-Neto et al. 2007). While investigating the occurrence of spiders on 70 individuals of *S. thomasiifolium*, we observed that all individuals were inhabited by *P. flava*, while none of their neighboring plants ($n = 80$) had spiders.

To understand this spider-plant interaction better, we addressed the following questions: 1) Is *P. flava* abundance related to *S. thomasiifolium* size? 2) Are insect predation rates by *P. flava* on *S. thomasiifolium* higher than predation rates by other predators (e.g., ants, other spiders) on neighboring plants without glandular hairs?

This study was performed in the Reserva Natural da Vale do Rio Doce (19°26′S, 40°03′W), 30 km north of the city of Linhares, state of Espírito Santo, a forest reserve covered mainly by Atlantic Forest vegetation. The reserve comprises 22,000 ha of forest, elev. 28–65 m above sea level, and a soil rich in sand. The weather is seasonal, with rainfall varying monthly from 30 mm in the dry season (June) to 226 mm in the wet season (January). Mean annual rainfall is 1320 mm. Temperature varies from 10° to 30° C (Jesus 1988). Samplings were done in July 1996 and July 1997, in a 4-ha area of common, nativo vegetation on sandy soil, dominated by cacti, bromeliads, herbs, and small shrubs (Peixoto & Gentry 1990).

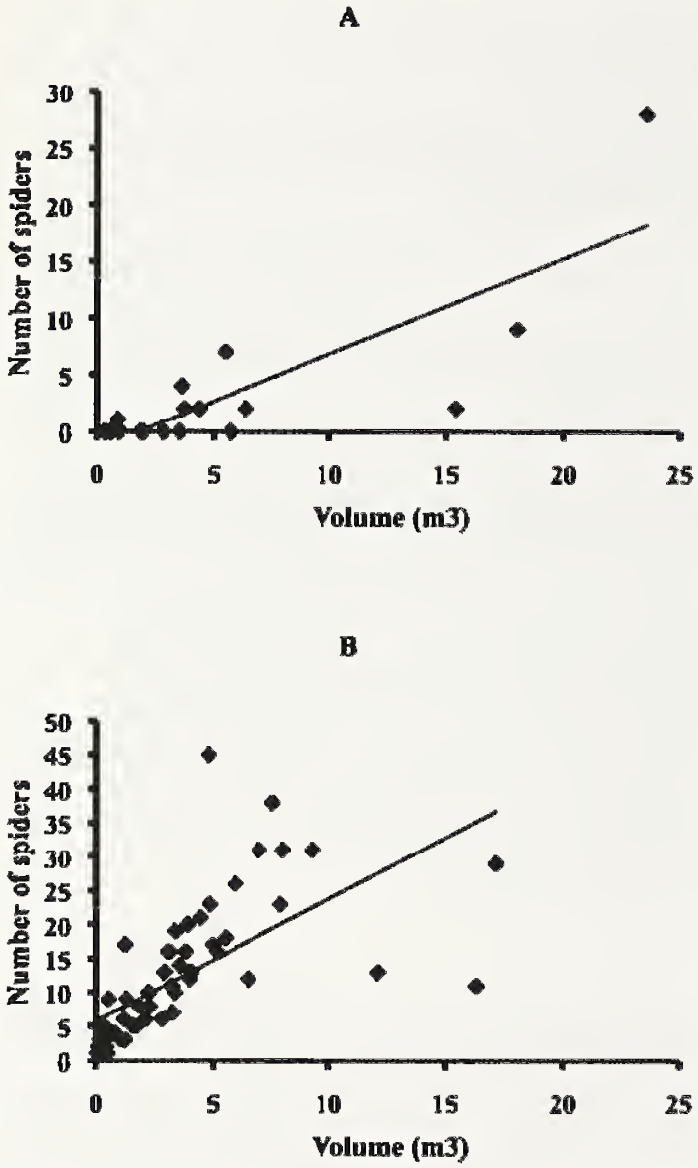

Figure 1.—Relation between *Peucetia flava* abundance and *Solanum thomasiifolium* size. A. 1996 ($n = 17$), $y = -1.543 + 0.840\,x$, $R^2 = 0.671$, $F = 30.569$, $P < 0.001$; B. 1997 ($n = 59$), $y = 5.710 + 1.810\,x$, $R^2 = 0.428$, $F = 42.618$, $P < 0.001$.

To verify whether the abundance of *Peucetia flava* correlates to the size of *Solanum thomasiifolium* plants, we estimated the size of plants in a 50-m random transect, in each year by measuring the maximum height of branches with leaves, the maximum canopy diameter, and the perpendicular length to this diameter. These measures were then multiplied to estimate plant size in cubic meters. The number of spiders was recorded on each plant by inspecting branches, leaves, and stems. This relationship was tested using linear regression.

We assessed the effect of spiders as bodyguards on *S. thomasiifolium* by using termite workers as herbivore models. We used termites instead of leaf-mining larvae because the former are thicker and less tender and, thus, easier to manipulate. In addition, once we attached termites to the surface they were unable to fly; thus, we could accurately judge predation rate. Fifteen plants of *S. thomasiifolium* and an equal number of similarly-sized neighboring plants without glandular trichomes were randomly selected in the study area. Each plant received ten termites randomly positioned on leaves of different branches, and after 30 min we recorded the number of individuals

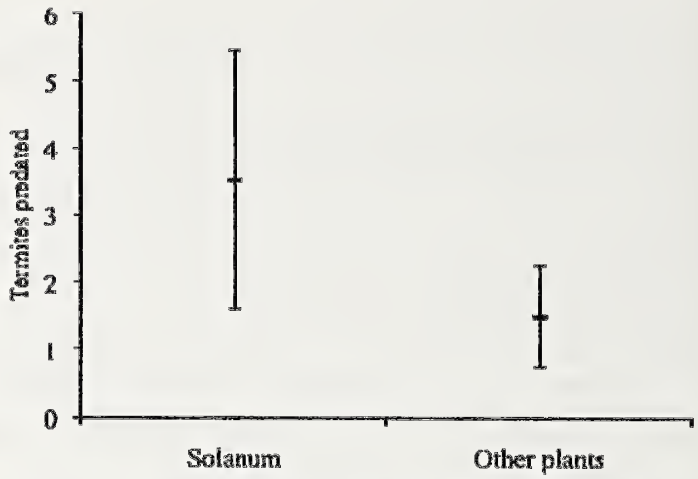

Figure 2.—Number of termites removed (mean ± SD) from *Solanum thomasiifolium* and other neighboring plants without glandular hairs.

removed. Termites were affixed with non-toxic white glue (Cascolar®) to plants without glandular hairs to prevent them from falling off. This glue had no influence on spider behavior (unpublished data, GQR). Additionally, previous studies have used similar methods for the same purpose and shown that this type of glue does not interfere with predatory (e.g., ant) behavior (e.g., Oliveira et al. 1987). All the plants were checked once during the experiment in an attempt to record predation events. The removal rate of termites from both plant types was compared using a *t*-test for independent samples.

To estimate the relationship between *P. flava* and herbivory on *S. thomasiifolium*, we calculated spider density and estimated leaf miner damage in July 1997. We evaluated leaf miner herbivory because it is the commonest kind of damage caused by herbivores on *S. thomasiifolium* in the study area (personal communication). Plants were randomly selected, and four branches of each individual were evaluated for leaf miner damage. The numbers of intact and damaged leaves were recorded for 20 plants, and the ratio between damaged and total leaves was calculated. Spider density was estimated as the ratio between the number of spiders and the plant size in cubic meters. The relationship was tested using linear regression. Data normality and homoscedasticity were verified. Logarithm transformations were applied when necessary prior to the analyses (Zar 1999).

Voucher specimens of the spiders collected (males and females) were deposited in the Araehnological Collection of the Laboratório de Artrópodes Peçonhentos, Instituto Butantan, São Paulo (accession numbers: IBSP 12982, IBSP 12940, IBSP 12891, and IBSP 12887). *Solanum thomasifolium* exsiccates were deposited at Universidade Estadual de Campinas Herbarium (UEC-Herbarium).

In 1996, we examined 17 *Solanum thomasiifolium* plants and found 57 *Peucetia flava* individuals. In 1997, we recorded 694 spiders on 59 plants. Linear regressions indicated that spider abundance was positively correlated with plant size during both sampling periods (Figure 1). Figure 2 displays the results of the termite removal experiment on the 30 observed plants, with significantly higher predation rates on *S. thomasiifolium* than for other plants ($t = 3.88$; $df = 28$; $P < 0.001$). *Peucetia flava* accounted for 11 of the 17 termite predation events observed on *S. thomasiifolium* (Figure 3). The other six events were recorded on plants without glandular trichomes. Those termites were removed by ants ($n = 4$) and salticid spiders ($n = 2$).

Larger plants had more spiders, probably because they provided more suitable habitat sites for the spiders. In a study of salticid-bromeliad association, Romero et al. (2007) showed that *Coryphasia monteverde* Santos & Romero 2007 inhabited large rosettes of

Figure 3.—*Peucetia flava* preying on termite placed on *Solanum thomasiifolium* (Photo G. B. J.).

Aechmaea distichantha and suggested that these spiders may actively select their microhabitats based on host plant size. Additionally, large plants may also represent a more suitable resource for many insects that constitute the main prey of the spiders. This hypothesis was proposed by Romero & Vasconcellos-Neto (2004a), who reported that another bromeliad-dwelling salticid, *Eustiromastix nativo* Santos & Romero 2004, had a similar microspatial distribution on two bromeliad species, possibly because larger plants have a higher probability of being visited by insects as a result of their large surface area.

Although we have no replicates or exclusion experiments demonstrating the influence of spiders on leaf miner density, observational and correlative data associated with the experiment using termites as herbivore models suggest that *P. flava* might act as an important plant bodyguard. The higher predation rates of the termite prey models on *S. thomasiifolium* compared to species without glandular trichomes suggest that *P. flava* is a very active insect predator, apparently surpassing the performance of sympatric ants and salticid spiders.

The role of *Peucetia* species in the reduction of the herbivore population and its destructive impact has already been already demonstrated in studies with other plants. For instance, Louda (1982) has shown that *P. viridans* (Hentz 1832) can decrease herbivore damage to its host plant, *Haplopappus venetus* (Asteraceae). In addition, Romero et al. (2008) recently showed that *P. flava* and *P. rubrolineata* maintain mutualistic relationships with their host plant, *Trichogoniopsis adenantha* (Asteraceae), the spiders removing herbivores from their host plants and the glandular hairs of the plants improving the spiders' growth by facilitating predation on adhering insects.

In this study, we have provided evidence that the relationship between *P. flava* and *S. thomasifolium* might be mutualistic. However, more definite conclusions should be obtained in the future by using spider exclusion experiments in the field.

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SHORT COMMUNICATION

Reducing scorpion fluorescence via prolonged exposure to ultraviolet light

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Abstract. A simple technique is presented for reducing the fluorescence of living scorpions by prolonged exposure to UV light. Scorpion's fluorescence peak can be eliminated by a 1-mo exposure to low intensity UV light. Although the fluorescence peak returns within 1 wk after removal from UV light exposure, the magnitude remains reduced. This technique potentially opens up new options for testing a variety of hypotheses about possible functions of scorpion fluorescence including potential effects on cuticle strength, visual responses, predation, cannibalism, and mating.

Keywords: Spectra, spectroscopy

There is no known function of scorpion fluorescence (Brownell 2001; Kloock 2008). Although it is certainly possible that fluorescence has no function, it is only by testing and falsifying potential functions that they can be eliminated from consideration. In order to test potential functions of scorpion fluorescence, having scorpions with reduced fluorescence could be a powerful tool. Several methods for reducing fluorescence exist, but those currently available are problematic. Kloock (2005) removed fluorescence from preserved scorpions by applying a varnish that blocked ultra-violet light (UV), and fluorescence can also be eliminated or reduced either by not supplying UV light or blocking it with filters. Use of coatings limits experiments because live, non-fluorescing scorpions cannot be used. In addition, coating scorpions introduces experimental complications, given the different chemicals needed to either block or allow fluorescence and secondary coatings to remove these effects dim the fluorescence of controls. Detecting fluorescence under natural conditions, if possible, requires very sensitive visual senses that scorpions may possess (Kloock 2008), but any dimming could prevent detection, and thus affect experimental attempts to demonstrate detection. Eliminating or blocking UV light with filters prevents fluorescence of living specimens, but also introduces a necessary experimental complication: effects of fluorescence and UV light cannot be separated by techniques that simply eliminate UV light. Given that scorpion eyes (Machan 1968) and their extra-ocular light sense (Zwicky 1970) are sensitive to both UV light and light near the fluorescence peak (~500 nm), this is a serious complication. Modifying scorpions so that they can be exposed to UV light without fluorescing can remove this problem, although possible behavioral and physiological side effects of any such manipulation could introduce new difficulties.

Anecdotal accounts suggest that long-term exposure to ultraviolet radiation may reduce scorpion fluorescence (Wankhede 2004). This has not previously been quantitatively demonstrated. I present here evidence that long-term exposure to UV light significantly reduces scorpion fluorescence.

Paruroctonus becki (Gertsch & Allred 1965) (Vaejovidae) were collected July–September 2008 in Kern County, California (voucher specimens deposited at the California Academy of Sciences), and housed in small, foam-plugged plastic vials. Sixteen females were randomly chosen from this population for the experimental manipulation and placed in small open-topped plastic arenas (13 cm long × 10 cm wide × 7 cm high) with 50 ml of soil collected from the same site as the scorpions. The soil helped to absorb and retain moisture, but was not deep enough to allow scorpions to bury themselves to escape irradiation. Scorpions were exposed 24 hrs/day for 32 days to two 40 W fluorescent blacklights (GE F40BLB) at a constant distance of 75 cm. This resulted in scorpions constantly receiving 11 $\mu W/cm^2$ of UV light energy (measured with a Mannix UV-340 light meter: range = 290–390 nm). Control scorpions were kept without significant UV light exposure. They were exposed to light from white fluorescent lights (≤ 1 $\mu W/cm^2$ UV) daily. Once a week, each scorpion was fed a single mealworm larva (*Tenebrio* sp.) and provided water by spraying the soil surface with a mister. I selected only females to simplify analysis and because females were more readily available. I see no reason to expect major differences between genders, but this should be tested in the future.

Within 2 wk, UV-exposed scorpions exhibited visibly reduced fluorescence, particularly on the dorsal surfaces. The thinner, more flexible portions of the exoskeleton (i.e., carapace and mesosoma) experienced a larger fluorescence reduction than the thicker regions (pedipalps, metasoma). After 32 days, fluorescence on the dorsal surfaces was no longer visible, though ventral surfaces and chelicerae still fluoresced dimly, in a pattern consistent with the effects of shading. At this point, the 12 surviving UV-exposed scorpions and 12 randomly selected control scorpions were measured with a reflectance spectrometer using a UV light source to stimulate fluorescence.

Emission spectra measurements were taken with a BW-Tek BRC111A CCD Spectrometer using BWSpec version 2.24 software with an integration time of 100 ms and 20 averages per sample. A single UV LED with peak emission at 390 nm and transmitted via a fiber-optic cable directly to the reflectance probe supplied excitation energy. A light-absorbing fabric (Edmund scientific #3060068) served as a dark reference, and I used the dark subtraction method for all measurements. The spectra were analyzed in raw form, without smoothing techniques (e. g., Fourier or running averages) applied to remove noise from the spectra.

All spectrum measurements occurred inside a light-tight box enclosing both the scorpion and reflectance probe. A squeeze cage, as described in Kloock (2008), immobilized scorpions during measurement. The reflectance probe was lowered until it touched the carapace at a point just behind the medial eyes. The order of measurements was randomized to reduce potential bias.

Fig. 1 provides a visual demonstration of the results of the manipulation. More importantly, spectrometer measurements showed large effects (Fig. 2, Table 1). The peak seen in each spectrum at ~400 nm (Fig. 2) results from reflection of the UV light source (peak emission at 390 nm) and is not the result of fluorescence. To avoid this artifact, all measurements were made over the range of 450–700 nm.

Thirty-two days of UV exposure produced a significant reduction in peak power (Table 1; *t*-test assuming unequal variance, $t = 8.88$, $P = 2.39 \times 10^{-6}$, $df = 12$) and a significant increase in the wavelength

Figure 1.—Photograph (grayscale) of two scorpions under black-light against a non-fluorescent white background. The scorpion on the left received prolonged exposure to UV light, the scorpion on the right did not. Preserved specimens were photographed due to the long exposure times required under these lighting conditions. Photo courtesy of Jason-Marc Mohamed. Used with permission.

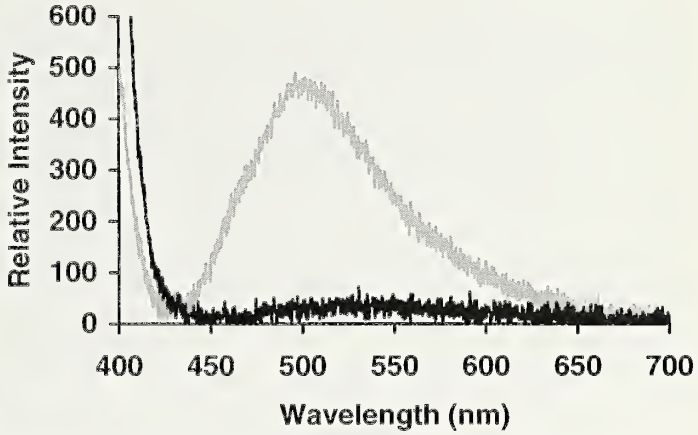

Figure 2.—Representative spectra in the visible range (400–700 nm) of a control scorpion (gray line) and a scorpion after 32 days of exposure to 11 μW/cm^2 UV light (black line). The intensity peak in both spectra as they approach 400 nm is eaused by refleetion from the light source, an UV LED with peak emission at 390 nm.

of peak power (*t*-test assuming unequal variance, $t = 4.48$, $P = 0.0007$, $df = 12$). The emission spectra for the UV-exposed scorpions show no clear peaks (Fig. 2). An *F*-test for variance ratios (Zar 1996) shows that the variance in the peak wavelength of the UV-exposed scorpions far exceeded the variance for control scorpions (UV-exposed scorpions $s^2 = 655$, control scorpions $s^2 = 19.0$, $F = 34.5$, $P = 6.46*10^{-7}$). This and the wide range of values (Table 1) indicate that the wavelength of peak power was essentially random for the UV-exposed scorpions.

After measurement, both UV exposed and control scorpions were placed in covered containers with soil and a shelter (a fragment of terra cotta pot) and maintained on a 13 hours white light: 11 hours dark cyclc, with feeding and watering schedules maintained as above. One week later, spectra were remeasured. Table 1 shows that in one week without UV exposure, significant recovery of fluorescence had occurred, with consistent peaks again evident in the spectra and no difference between groups in wavelength of peak power (*t*-test assuming unequal variance, $t = 1.32$, $P = 0.204$, $df = 12$). Although the relative intensity of the fluorescence was still significantly reduced compared with controls (*t*-test assuming unequal variance, $t = 4.60$, $P = 6.06 \times 10^{-4}$, $df = 12$.), fluorescence intensity increased significantly within the UV-exposed group over the one-week recovery period (*t*-test assuming unequal variance, $t = 3.56$, $P = 1.56 \times 10^{-4}$, $df = 12$). Significant differences in the variance between controls and UV-exposed scorpions still existed for peak intensity ($F = 35.2$, $P = 5.79 \times 10^{-7}$) and for peak wavelength ($F = 3.28$, $P = 0.030$). Preserved UV-reduced specimens showed no signs of recovery, indicating that active metabolic processes are responsible for recovery. Determining just what those proccsses are will require further study.

Photobleaching, the loss of fluorescence due to prolonged exposure to excitation wavelengths, is commonly encountered in fluorescence microscopy, which focuses on methods of preventing it (Deschenes & Vanden Bout 2002). Photobleaching is probably caused by a variety of different mechanisms (Georgakoudi et al. 1997), so more detailed study will be needed to determine a mechanism of the photobleaching observed here. Two molecules responsible for scorpion fluorescence have been identified: ß-carbolinc (Staehel et al. 1999) and 4-methyl-7-hydroxycoumarin (Frost et al. 2001), which may aid future investigations into this phenomenon. Similarly, the mechanism of recovery has not yet been investigated, and possible side effects of the treatment, including the potential of rctinal or other tissue damage and effects on behavior of long-term exposure, need to be investigated and controlled in any future experiments using this technique.

With this important caveat, the ability to reduce fluorescence potentially opens up a broad variety of new experiments. For example, Camp & Gaffin (1999) and Blass & Gaffin (2008) suggest that fluorescence may function as a light amplifier, or aid in detecting UV light. If true, then scorpions with reduced fluorescence should display an altered light avoidance response. Tests can also be designed to test ecological hypotheses of function (summarized in Kloock 2008). For example, experiments could be designed to test whether scorpions with reduced fluorcseenee experienee different levels of predation, cannibalism, prey capture or mating success than fluorescent scorpions under diffcrent lighting conditions in both natural and laboratory settings.

It is also possible that fluoreseence is a byproduct of a molecule whose primary function is unrelated to fluorescenee itself. For example, Stachel et al. (1999) suggested that the fluorescent molecule functions in sclerotization. To test this hypothesis, experiments can be designed to test the effects of fluorescence reduction on cuticle strength. Similarly, the fluorescent molecule may function in reducing water loss (Lourenço & Cloudsley-Thompson 1996), and experiments could be designed to determine the effect of fluorescence reduction on the rate of water loss.

Caution must be exercised in experimental design because we have, as yet, no information on side effects of the technique of fluorescence

Table 1.—Data from fluorescence spectra of control and UV-exposed scorpions after 1 mo of exposure, and repeated after 1 wk of recovery. $n = 12$ for each treatment. Peak values determined over the range 450–700 nm.

Measurement	Treatment	Mcan peak relative intensity (SE)	Mean wavelength of peak intensity, nm (SE)	Range: wavelength of pcak intensity, nm
After exposure	Control	608 (120)	501 (1.26)	496–511
	UV-exposed	72.1 (10.2)	535 (7.39)	497–576
After recovery	Control	482 (77.2)	501 (2.16)	492–519
	UV-exposed	122 (13.0)	508 (3.91)	490–533

reduction itself on scorpion behavior or physiology, which could complicate future experiments. The challenge of experimental design using this technique will be to adequately control for potential side effects of fluorescence reduction. One way to accomplish this will be to cross UV presence and fluorescence reduction: if fluorescence reduction has an effect unrelated to fluorescence, similar differences should be observed between fluorescence-reduced and control scorpions regardless of the presence of UV light. Of course, the details of any experiment may require more complicated controls to be developed. Provided adequate controls are used, this technique makes possible future experiments designed to determine whether or not scorpion fluorescence (or the fluorescent molecules) serves specific functions. Even if fluorescence serves no function, such experiments can enhance our understanding of scorpion ecology, physiology, and behavior.

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SHORT COMMUNICATION

Presence of *Vaejovis franckei* in epiphytic bromeliads in three temperate forest types

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Abstract. Reports of scorpions on epiphytic bromeliads in temperate forests are scarce. Here we present some ecological aspects of this animal-plant interaction in three different types of temperate forests (pine, pine-oak and oak forest) in Oaxaca, Mexico. From 2005 to 2007, we collected 373 bromeliads belonging to 10 species, and each plant was defoliated in search of scorpions. We found 35 individuals of *Vaejovis franckei* Sissom 1989 in 19 bromeliads: 22 specimens in *Tillandsia carlos-hankii* with 21% occupancy and an average abundance of 2.1 ± 1.9 individuals/plant; 12 specimens in *T. prodigiosa* (10% occupancy, average abundance = 1.6 ± 0.6) and one specimen in *T. calothyrsus* (3% occupancy, average abundance = 1 ± 0.0). Pine-oak forest had 29 individuals; pine forest, 4 individuals; and oak forest, 2 individuals. Percentage of occupancy differed among localities, while average abundance remained the same. *Vaejovis franckei* preferred *T. carlos-hankii* and pine-oak forest, which was correlated with the percentage of occupancy but not with the average abundance.

Keywords: Phytotelmata, Mexico

The presence of scorpions in tank-type bromeliads has been widely reported (Lucas 1975; Richardson 1999; Santos *et al.* 2006); however, most studies of scorpions on bromeliads have taken place in tropical forests, whereas research on bromeliads in temperate forests is scarce (Lucas 1975; Ochoa et al. 1993; Sissom 2000).

The present study sought to evaluate different ecological aspects of scorpions living in tank-bromeliads in three types of temperate forests. The study was carried out in Santa Catarina Ixtepeji in the state of Oaxaca, Mexico, located at 17°09′–17°11′N and 96°36′–96°39′W. Its climate varies with the altitude, and ranges from temperate to cold sub-humid with summer rains. The mean annual temperature and precipitation are 14° C and 1000 mm, respectively (INEGI 1998). Three sampling sites were selected: Peña Prieta (2870 m), a pine forest; La Petenera (2547 m), a pine-oak forest; and El Cerezal (2300 m), an oak forest. From 2005 to 2007, we sampled 373 bromeliads belonging to ten species. Bromeliads were transported to the laboratory where each plant was defoliated, and each leaf was carefully inspected. Scorpion specimens were preserved in 70% alcohol. We used the taxonomic keys of Hoffmann (1931) and Stockwell (1992) to identify the scorpions. Collected specimens have been placed in the Colección Nacional de Arácnidos in the Instituto de Biología (IB-UNAM) in Mexico City.

A total of 35 *Vaejovis franckei* Sissom 1989 was found in 19 of the 373 sampled bromeliads. We only found scorpions on *Tillandsia prodigiosa* (Lem) Baker 1889, *Tillandsia carlos-hankii* Matuda 1973 and *Tillandsia calothyrsus* Mez 1896. No specimens were found in *Viridantha plumosa* (Baker) Espejo 2002 (n = 22), *Catopsis berteroniana* (Schult. & Schult.f.) Mez 1896 (n = 17), *Tillandsia macdougallii* L.B. Smith 1949 (n = 39), *T. oaxacana* L.B. Smith 1949 (n = 50), *T. magnusiana* Wittm. 1901 (n = 10), *T. bourgaei* Baker 1887 (n = 30), or *T. violaceae Baker*, 1887 (n = 38).

The greatest number of scorpions was found in *T. carlos-hankii* (24 individuals), followed by *T. prodigiosa* (10 individuals) and *T. calothyrsus* (1 individual) (see Table 1). The percentage of occupancy differed among species (X^2_2 = 7.60, P = 0.022, n = 167), whereas the average abundance of scorpions per plant was the same (*T. prodigiosa* = 1.6 ± 0.6, *T. carlos-hankii* = 2.1 ± 1.9 and *T. calothyrsus* = 1.0, ANOVA, $F_{2,17}$ = 1.03, P = 0.3).

Pine-oak forest (La Petenera) produced the greatest abundance of *V. franckei*, with 29 individuals, followed by pine forest (Peña Prieta) with 4 individuals and oak forest (El Cerezal) with 2 individuals. The percentage of occupancy differed among sampling localities (8% in Peña Prieta, 24% in Petenera and 3% in El Cerezal; X^2_2 = 14.88, P = 0.006, n = 167), while the average abundance of scorpions per bromeliad did not differ significantly among sites (Peña Prieta = 1.3 ± 0.6, Petenera = 2.1 ± 1.3 and Cerezal 1.0; ANOVA, $F_{8,16}$ = 1.1, P = 0.4). The average abundance of scorpions by bromeliad (r = 0.18, P = 0.9) was not correlated with the size of the bromeliad, although the percentage of occupancy was significantly related to it (Kendall Tau = 0.154, P = 0.018, n = 107)

Most studies of arthropods living inside bromeliads do not show specificity for particular bromeliad species (Richardson 1999; Ospina-Bautista et al. 2004; Liria 2007), even though some arthropods show a strong preference for certain species of bromeliads (Quevedo & Vasconcellos-Neto 2005; Osses et al. 2007). In our case, *V. franckei* preferred *T. carlos-hankii*. This preference could be related to the architecture and/or color of the plant; e.g., *T. carlos-hankii* has green leaves with a purple base. In contrast, *T. prodigiosa* possesses green pale leaves. These differences could promote microclimatic conditions that favor the presence of scorpions, although further research is required to reach such a conclusion.

Although bromeliad size did not correlate with scorpion abundance, there may be a minimum size of bromeliad that can support scorpions, since scorpions were absent in three small species (*V. plumosa*, *T. macdougallii* and *T. magnusiana*) that do not form a water tank. Size limits the amount of water and leaf litter that accumulates inside these bromeliads, resulting in a decrease in arthropod species richness and abundance (Benzing et al. 2000), including many species that scorpions prey upon. Other studies have also shown that small bromeliads have lower abundance and species richness of arthropods that scorpions feed on, potentially resulting in lower scorpion abundances in these bromeliads (Ospina-Bautista et al. 2004; Franco 2008). Scorpions were not found in *T. oaxacana* either, probably because of the plant's small size (17–30 cm in height) and its small tank, which retains a maximum of 300 ml of water, compared to 1400 ml for *T. carlos-hankii* (Franco 2008). Bromeliad size might also directly limit the presence of scorpions, as these arthropods are relatively large, measuring up to 6 cm in length. Space within the bromeliads might thus be inadequate to provide a refuge for scorpions. Accordingly, we observed scorpions only on the larger bromeliad species (between 50 and 75 cm in height) such as *T. prodigiosa*, *T. carlos-hankii* and *T. calothyrsus*.

Table 1.—Presence of *Vaejovis franckei* Sissom 1989 on different bromeliad species in three temperate forests in Santa Catarina Ixtepeji. a = number of sample plants, b = number of plants with scorpions, c = scorpion abundance.

Bromeliad species	Pine forest			Pine-oak			Oak		
	a	b	c	a	b	c	a	b	c
Tillandsia prodigiosa (Lem) Baker, 1889	0	0	0	40	5	9	37	1	1
Tillandsia carlos-hankii Matuda, 1973	40	3	4	18	9	20	0	0	0
Tillandsia calotlyrsus Mez, 1896	0	0	0	0	0	0	32	1	1

Scorpions show high specificity for certain environmental conditions (Hoffmann 1931; Lourenço & Sissom 2000; Flórez 2001; Prendini 2001). Physical conditions in the pine-oak forest site are probably the most ideal for *V. franckei*, given that it was most common in this forest type (29 individuals). The low abundance of *V. franckei* in the pine forest may explain why this scorpion was not found in *T. violaceae* in this forest type, despite the plant's suitability for the establishment of scorpions. Nonetheless, at least one other species of scorpion, *C. flavopictus*, has been found in this plant species in a cloud forest in Chiapas (Lucas 1975).

Although tank-type bromeliads appear to be a very attractive habitat for scorpions because they represent a potential source of food and refuge, in this study they colonized only a small percentage of the bromeliad specimens (9%). This result agrees with observations by Santos et al. (2006), who also reported a low percentage of occupancy (13%) for *Tityus neglectus* Mello-Leitão in four tank-type bromeliad species. Such low occupancy levels may be due to overall low scorpion densities, as reported across most types of vegetation (Bradley & Brody 1984).

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SHORT COMMUNICATION

Taxonomic notes on the genus *Microfilistata* (Araneae: Filistatidae), with a description of a new species from Turkmenistan

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Abstract. *Microfilistata ovchinnikovi* new species (Araneae: Filistatidae), the second member of this formerly monotypic Central Asian genus, is described from Kyzyl-Dzhar Ravine, Southern Turkmenistan. The genus is redescribed and referred to the Filistatidae insertae sedis.

Keywords: spider, Filistatidae, taxonomy, Central Asia

The genus *Microfilistata* was based on a single incomplete male specimen from Uzbekistan (Zonstein 1990). According to Gray (1995), palpal characters of the genus show some resemblance to those of the filistatine genera but relationships of *Microfilistata* remain unresolved. The occurrence of the second congener in Turkmenistan, represented also by females, seems to be helpful to provide the genus with a complete description. The type series, including the holotype of this new species, is kept in Department of Zoology, Tel-Aviv University, Israel (TAUI). Measurements are expressed in millimeters, except eye diameters/interdistances shown as the ratio of microscope scale units at 100x magnification. Abbreviations: AME, ALE, PLE, PME = anterior median and lateral, posterior lateral and median eyes; ALS, PMS, PLS = anterior lateral, posterior median and lateral spinnerets, respectively.

Microfilistata Zonstein 1990

Microfilistata Zonstein 1990:51; Gray 1995:80.

Type species.—*Microfilistata tyshchenkoi* Zonstein 1990 by original designation and monotypy.

Diagnosis.—Males belonging to *Microfilistata* can be distinguished from other male filistatids by their enclosed palp. Conspecific females are characterized by unusually (for female filistatids) long and slender palps and legs covered with a reduced number of long setae.

Redescription.—Small pale-colored filistatids with body length of 1.6–1.7 mm in males and 2.5–2.7 mm in females; legs and abdomen without pattern. Carapace domed, broad-oval, wide-rounded anteriorly. Thoracic fovea absent. Clypeus short and steeply inclined with few stout erect bristes. Eye tubercle low. ALE > PLE ≈ PME > AME. Chelicerae small, downward-directed; cheliceral furrow and fang very short; prolateral lamina small but distinct. Sternum subcircular, sigillae not evident (Figs. 3, 6). Labium slightly wider than long. Maxillae trapezoidal. Pedipalps and legs long and slender, both in males and females. Male palpal tibia long, cylindrical. Cymbium long, with apical extension entirely covering the tegulum. Embolus sharpened, more or less curved. Leg formula: 1423. Sexual dimorphism in leg length weakly developed. Legs covered with rather sparse, long setae. All femora with one weak dorsoproximal spine. Leg tarsi ascopulate, long and entire, without pseudosegmentation. Short calamistrum represented by a few curved and flattened setae on raised keel. Paired tarsal claws narrow and curved, with a single row of few weak teeth. Unpaired claw weakly curved, edentate. Female spermathecae divided. Spinneret group small and located closer to abdomen tip. Cribellum small, bipartite, trapezioidal. ALS and PLS with thickened setae, PMS with one probably paracribelar gland spigot (PS).

Notes.—Like the filistatine genera, *Microfilistata* possesses a short and narrow calamistrum confined to the metatarsal crest in females (shown as a filistatine synapomorphy by Ramírez & Grismado, 1997) and a long cylindrical cymbium with a highly coiled ejaculatory duct in males. On the other hand, the genus resembles in some aspects the members of the Prithinae: in both cases the thoracic fovea appears to be undeveloped, only one PS is present (indicated by Ramirez & Grimaldo, op. cit., as a prittine synapomorphy), and male tarsi are entire, not pseudosegmented, cracked or bent; although at least some of the mentioned characters in *Microfilistata* could be explained rather by a miniaturize size of the congeners. Hence, the characters of *Microfilistata* are found to be doubtful, and the genus is referred to the Filistatidae incertae sedis until a further knowledge on Asian filistatids is attained.

Microfilistata ovchinnikovi new species
(Figs. 1–8)

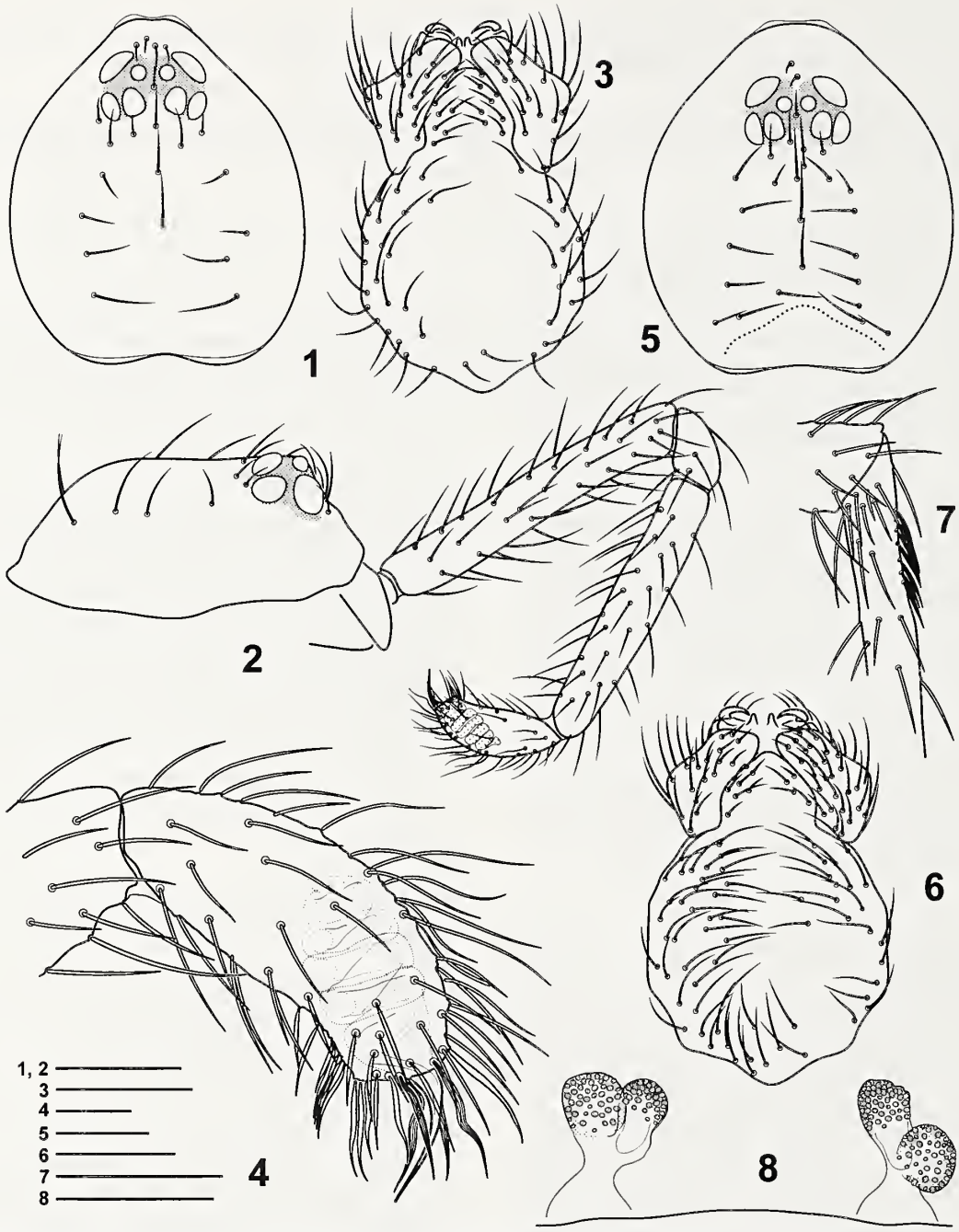
Types.—Male holotype and 3 female paratypes from Kyzyl-Dzhar Ravine, 580–620 m above sea level, SE border of Badkhyz Plateau, Turkmenistan (35°48′N, 61°53′E), 11 April 1993, S.V. Ovchinnikov (TAUI).

Etymology.—The specific name is a patronym in honor of the late Central Asian arachnologist Mr. Sergei Ovchinnikov, the collector of the species and a good friend of mine.

Diagnosis.—Differs from *M. tyshchenkoi* by a different eye ratio (AME:ALE 1:2 vs. 1:3 in the latter species) and by having a more thin, tapering and spirally twisted embolus. Any structures resembling palpal conductor absent.

Description.—Male (holotype): Total length 1.61; whole spider pale yellowish-brown save for the dark brown eye tubercle and prolateral lamina, and brownish setae, chelicerae and tarsal claws; abdomen light yellowish-gray. Carapace (Fig. 1): 0.68 long, 0.59 wide. Eye tubercle (Fig. 2) low. Ratio of AME, ALE, PLE, PME: 5, 10, 7, 6. Interdistances: AME–AME 2, ALE–AME 1, ALE–PLE 1, PLE–PME 1, PME–PME 3. Labium and sternum as shown on Fig. 3. Measurements (length): palp: femur 0.66, patella 0.16, tibia 0.57, cymbium 0.28; legs 1–4: femora: 1.22, 0.91, 0.86, 1.04; patellae: 0.25, 0.23, 0.22, 0.23; tibiae: 1.24, 0.77, 0.62, 1.17; metatarsi: 1.12, 0.83, 0.78, 1.07; tarsi: 0.70, 0.52, 0.45, 0.53. Palps lack spines. Leg spination: femora: I d1–0–0, p 0–0–1, r 0–0–1, II-IV d1–0–0; tibiae: I–II d1–0–0, p 0–0–1, v 0–1–0, r 0–0–1; III–IV d1–0–0, p 0–0–1, r 0–0–1; metatarsi: I p 0–1–1, r 0–1–1; other segments lack spines. Palpi as shown on Figs. 3, 4. Paired tarsal claws with 3–4 weak teeth, unpaired claw bare.

Female (paratype): as in male, except as noted. Total length 2.70. Carapace (Fig. 5): 0.94 long, 0.84 wide. Ratio of AME, ALE, PLE, PME: 5.5, 10, 9, 8. Interdistances: AME–AME 2, ALE–AME 2, ALE–PLE 1, PLE–PME 1, PME–PME 3. Measurements (length): palp: femur 0.65, patella 0.22, tibia 0.48, cymbium 0.53; legs 1–4: femora: 1.17, 0.95, 0.91, 1.02; patellae: 0.33, 0.32, 0.23, 0.33; tibiae: 1.23, 0.86, 0.79, 1.04;



Figures 1–8.—*Microfilistata ovchinnikovi* sp. n., male (1–4) and female (5–8). 1, 5. Dorsal view of carapace; 2. Lateral view of carapace, chelicera and palp; 3, 6. Ventral view of chelicerae, labium, sternum and maxillae; 4. Lateral view of male palpus; 7. Lateral view of calamistrum; 8. Ventral view of spermathecae. Scale for 1–3, 5–7 = 0.25 mm; for 4 and 8 = 0.1 mm.

metatarsi: 1.10, 0.81, 0.76, 1.00; tarsi: 0.74, 0.53, 0.48, 0.51. Femora I–IV with one basodorsal spine. Labium and sternum, calamistrum and spermathecae are as shown in Figs. 6, 7, and 8, respectively.

Distribution and habitat.—Known only from the type locality. Spiders inhabit cavities and cracks in rock cliffs and escarpments of the ravine.

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SHORT COMMUNICATION

Evidence for multiple paternity in broods of the green lynx spider *Peucetia viridans* (Araneae: Oxyopidae)

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Abstract. In the green lynx spider *Peucetia viridans* (Hentz 1832), the two openings of a mated female's epigynum are often sealed by copulatory plugs, sometimes with the two-pronged distal portion of the paracybium of a male palpus inserted in each opening and embedded in the plugs. The presence of copulatory plugs and paracymbia may prevent further mating by the female. However, not all mated females exhibit these structures, perhaps allowing some *P. viridans* females to mate with more than one male, despite the assertion of Whitcomb & Eason (1965) that females only mate once. We investigated this possibility by surveying the extent of multiple paternity in field-collected *P. viridans* broods from southern California. For adult females and their egg sacs, we determined the aspartate aminotransferase genotype for each mother and her spiderlings using allozyme electrophoresis in order to assess whether the progeny data best fit with a single male as the father. Two broods exhibited clear evidence of multiple paternity, verifying that multiple mating by females is possible in this species. Although most mothers of single paternity broods had one or both epigynal orifices blocked, some had no blockage at all, while the two mothers of multiple paternity broods had some kind of blockage to one or both orifices, suggesting that neither plugs nor inserted paracymbial processes are assoeiated with a reduction in female remating.

Keywords: Multiple mating, polyandry, molecular marker, copulatory plug, paracymbial process

The green lynx spider *Peucetia viridans* (Hentz 1832) is the largest and commonest member of the family Oxyopidae, with a distribution throughout the southern United States, Mexico, and Central Ameriea (Brady 1964). It is a cursorial hunter that forages on prey commonly found on plants (Arango et al. 2000). Although little studied up to 1960, *P. viridans* has been the sole or partial focus of at least 25 papers since then, making it one of the best-characterized hunting spiders in North America.

While much is now known about the reproductive biology of *P. viridans*, one question that remains unresolved is whether adult females ever remate in the wild. This is especially significant given that *P. viridans* and *P. longipalpis* F.O. Pickard-Cambridge 1902 are the only oxyopids known to produce copulatory plugs (Suhm et al. 1996), structures which are commonly thought to delay and/or reduee the probability of female remating (Eberhard 1996). Brady (1964) was the first to note the presence of plugs in *P. viridans* females, as he found that the two openings of a mated female's epigynum were usually plugged with a hard, black material, often with the two-pronged distal portion of the paracybium of a male palpus inserted in each opening and embedded in the material. Brady stated that the black material must be deposited during or immediately after insemination, a suggestion possibly corroborated by Whitcomb & Eason's (1965) observation during a laboratory study of a large drop of shiny liquid on the epigynum of a female immediately following copulation that later disappeared.

Brady (1964) reasoned that the plugging of the female epigynum and the loss of the male paracymbial process should prevent further matings by both female and male (although since males possess two palpi, an individual male could potentially mate twice). However, in their study of mating behavior in *P. viridans*, Whitcomb & Eason (1965) found that each mating episode involves numerous copulations between female and male with both palps being inserted alternately into the epigynal openings. They also found that males mated freely on successive days, with one male having mated with three different females over consecutive days. In contrast, an individual female would only mate with one male and would actively reject subsequent male suitors. Of course, if the copulatory plug in *P. viridans* is indeed a device for impeding access to subsequent males as suggested by Brady (1964), perhaps assisted in this role by broken-off male paracymbia, why it is necessary if females never remate is unelear. However, since females of many species may be more reluctant to remate in captivity than in nature (Eberhard 1996), Whitcomb & Eason's (1965) assertion that female *P. viridans* mate only once may not be universally true, as it was based on laboratory observations.

Whitcomb & Eason (1965) also reported on the frequency of the copulatory plug, as did Exline & Whitcomb (1965), who also provided data on the frequency of inserted paracymbia. Whiteomb & Eason (1965) found plugs in all mated females they examined, but not in any virgin females. In contrast, Exline & Whitcomb (1965) stated that not all mated females exhibit this covering, noting that it can be easily removed and is probably sometimes lost during egg-laying. They found that in a sample of approximately 20 mated females, 10 had at least one male paracybium embedded in the plug. Among the remaining 10, the plug was missing altogether or did not contain a paracybium. Like Exline & Whitcomb (1965), we have found that the presence of plugs and paracymbia in the epigyna of mated *P. viridans* females is variable in both laboratory-mated and wild females, with almost half of a field-collected sample of females with egg saes from southern California (n = 54, 2004) having neither plugs nor paracymbial processes in their epigynal orifices (unpublished data). Thus, since plugs and paraeymbia are often absent as obstacles in the epigyna of mated *P. viridans* females, some females may be physically capable of mating with more than one male. Given this possibility, the purpose of this study was to search for cases of multiple paternity in field-collected *P. viridans* broods using a genetic approach, since such broods would result from individual females having had more than one male sexual partner.

From October through December 2007, we collected 29 adult *P. viridans* females with their respective egg sacs from six sites in southern California (population abbreviation and sample size are

indicated in parentheses): Los Angeles Co.—Kenneth Hahn State Recreation Area (HSR, 18), Yvonne Burke Sports Complex (HBF, 2), Ernest Debs Regional Park (DEB, 3), Robert Bernard Biological Field Station, Claremont (BFS, 1); San Diego Co.—Crest Canyon Preserve, Del Mar (CC, 4), Carmel Valley Road, Del Mar (CVR, 1). In the laboratory, we assigned adult females and their respective egg sacs unique identification numbers. Each female was microscopically examined for the presence of epigynal plugs, their condition noted, and any retained male paracymbial processes were recorded. Females were then frozen at −85° C pending genetic analysis. The egg sacs were maintained separately in small plastic tumblers until spiderlings had emerged from each sac, typically within 2–4 weeks of collection. Up to 60 randomly chosen spiderlings from each brood were then placed individually into 1.5 ml microcentrifuge tubes marked to match the identification number of their respective mothers. These brood samples were also then frozen at −85° C.

We used allozyme electrophoresis as our molecular paternity assessment technique, given its cost-effectiveness for large samples. Procedures for horizontal starch gel electrophoresis generally followed Ramirez (1990) and used gels that were 12.5% starch (StarchArt). We homogenized individual spiderlings directly in their 1.5 ml microcentrifuge tubes along with 20 µl of deionized water using a hand-held microcentrifuge tube pestle. This homogenate material was centrifuged at 484 G's at 0–4° C for 5 min to separate extracted proteins from cellular debris and was then frozen at −85° C until needed for gel loading. The adult females were homogenized individually in 15 ml Corning centrifuge tubes with an approximately equal volume of deionized water using a motorized grinding pestle. These homogenates were centrifuged at 12,100 G's at 0–4° C for 15 minutes. Following centrifugation, the relatively greater volume of liquid supernatant for each female was transferred into multiple 0.5 ml microcentrifuge tubes, which each contained sufficient material for one gel run; these tubes were then frozen at −85° C pending gel loading. Since our study involved destructive processing of the specimens, no vouchers were retained.

During a related study, we found that five loci are regularly polymorphic in *P. viridans* populations (aspartate aminotransferase, AAT-1,-2, E.C. 2.6.1.1; glucosephosphate isomerase, GPI, E.C. 5.3.1.9; lactate dehydrogenase, LDH, E.C. 1.1.1.27; phosphoglucomutase, PGM, E.C. 2.7.5.1) (Commission on Biochemical Nomenclature 1979). Unfortunately, three of these loci (AAT-2, GPI, PGM) presented problems of poor resolution of multiple alleles and/or inconsistent staining during test gel runs, and so were not used. The two remaining loci (AAT-1, LDH) are both diallelic systems that resolve well in adults, but since LDH could not be consistently scored in spiderlings during preliminary testing, probably due to a low level of enzyme concentration in their homogenates, only AAT-1 (a cationic locus) was used in this study. The recipe for AAT was based on Manchenko (1994), and it was resolved using the Continuous Tris-Citrate I buffer system of Selander et al. (1971). Females and their respective brood samples were run side-by-side on the same gels to aid in recognizing band homologies during genotype assignment. Agreement between observed genotypic proportions and Hardy-Weinberg expectations was evaluated for the adult females by calculation of exact significance probabilities using the BIOSYS-2 (Black 1997) computer program.

To determine whether a single male was unlikely to account for the genetic diversity of a female's brood, we used a significant deviation from a Mendelian ratio among progeny genotypes as our criterion. We identified the paternal alleles present in each brood by inspection of the progeny and maternal genotypes and then used this single paternal genotype along with the maternal genotype to determine an expected Mendelian progeny distribution. We tested the observed genotype numbers against the expected numbers using a Chi-square test with Yates continuity correction for small sample size (X^2_c), as implemented in the E-Z Stat 1.0.1 (Towner 1999) statistical analysis program.

The frequencies of the A and B alleles at the AAT-1 locus were 0.862 and 0.138, respectively, for the adult females and genotype numbers did not differ significantly from Hardy-Weinberg expectations. The 29 broods of *P. viridans* yielded 1337 spiderlings, which were genotyped at the AAT-1 locus along with their respective mothers (mean brood sample = 46, range = 15–60). In 17 broods, the mothers and spiderlings were all of the same genotype (AA), making it impossible to test for deviations from Mendelian ratios among progeny genotypes. The 12 remaining broods contained two or three offspring genotypes and among these, two (HSR-160, HSR-179) showed significant deviations from expected Mendelian ratios (Table 1), indicating that a single male was unlikely to account for the observed ratios in each case. Since multiple mating episodes involving males of the same genotype cannot be detected using a single diallelic locus, the frequency of multiple mating (2/12 = 16%) reported here may be an underestimate, especially given the limited number of broods analyzed. Nonetheless, we have verified that *P. viridans* females sometimes mate with more than one male, contrary to Whitcomb & Eason's (1965) assertion that females do not remate.

Among the 12 females whose broods contained multiple genotypes suitable for paternity assessment, the presence and state of the epigynal plug was quite variable, as was the presence of male paracymbial processes (Table 1), consistent with the findings of Exline & Whitcomb (1965) and our prior observations in southern California. Of the 10 females whose broods provided no evidence of multiple paternity, 7 (CC-17, DEB-330, DEB-343, HSR-141, HSR-143, HSR-144, HSR-176) possessed a complete copulatory plug in one or both of their orifices, sometimes accompanied by a paracymbial process, while the remaining three (CC-15, HSR-148, HSR-177) had neither plugs nor paracymbial processes in their orifices (Table 1). As for the two females whose brood genotypes indicated multiple paternity (HSR-160, HSR-179), HSR-160 had complete plugs in both orifices and HSR-179 had a partial plug in one orifice and no plug in the other, while neither possessed paracymbia in their epigynal orifices (Table 1). Thus, although most females classified as "singly inseminated" had one or both of their orifices blocked, three had no blockage at all, while both of the multiply inseminated females had some kind of blockage to one or both orifices.

In this study, we found clear evidence for the occurrence of multiple paternity in *P. viridans* offspring from southern California. At a minimum, 16% of the broods that were suitable for paternity assessment had been multiply sired. The fact that some level of female remating and multiple paternity is possible in this species indicates that sperm competition is a component of sexual selection in *P. viridans*. A widespread adaptive response for paternity assurance given sperm competition is the formation of copulatory plugs (Wigby & Chapman 2004), so their production in *P. viridans* is understandable. Moreover, since parts of the male palp often break off within the female during mating in several groups of spiders (Eberhard 2004; Huber 2005), where they can act as impediments to sperm transfer by subsequent males (e.g., Fromhage & Schneider 2006), the paracymbial process of *P. viridans* males may similarly serve as a copulatory plug when lodged in a female. However, both copulatory plugs and palpal structures in the female genital tract are less than 100% effective at preventing female remating in many spiders (references in Huber 2005; Schneider et al. 2005), perhaps partly due to female efforts to counteract male monopolization if they can benefit from polyandry (Hosken et al. 2009). More generally, plugs of any sort are not expected to be absolute barriers since selection would then favor male avoidance of nonvirgin females and plugging would be selected against (Eberhard 1996). As for *P. viridans*, while we cannot know how many of the plugs and paracymbial processes we observed were the result of first matings, the fact that both multiply inseminated females had some kind of blockage to their epigynum and three females classified as singly inseminated had no blockage at all

Table 1.—Distribution of AAT-1 genotypes among *Peucetia viridans* spiderlings from 12 brood samples, along with maternal epigynal configurations. The expected brood genotype distributions (in parentheses) assume Mendelian inheritance of two alleles (A, B) at the AAT-1 locus and appropriate genotypes for the unknown male parents based on the paternal alleles evident in each brood. The Chi-square test with Yates correction (X^2_c) evaluates the hypothesis that a single male inseminated each female; superscript ^{M} designates females determined to be multiply inseminated. Scoring of the female epigyna is the same for both the left and right epigynal orifices (LO, RO): ○ = copulatory plug absent; ● = complete plug present; ◎ = partial plug present; ⌊ or ⌋ = male paracymbial process in left or right orifice.

Female	Female genotype	Presumed male genotype	Spiderling genotypes AA	AB	BB	X^2_c	P	Epigyna LO	RO
CC-15	AB	AA	32 (30)	28 (30)	---	0.150	0.699	○	○
CC-17	AA	AB	21 (20)	19 (20)	---	0.025	0.874	●	●
DEB-330	AA	AB	16 (15.5)	15 (15.5)	---	0.000	1.000	●	○
DEB-343	AB	AB	18 (15)	28 (30)	14 (15)	0.546	0.761	●	○
HSR-141	AA	AB	34 (29.5)	25 (29.5)	---	1.085	0.298	●	●
HSR-143	AB	AA	10 (12.5)	15 (12.5)	---	0.641	0.423	◎	●
HSR-144	AB	AA	33 (30)	27 (30)	---	0.417	0.519	●	●
HSR-148	AB	AA	32 (30)	28 (30)	---	0.150	0.699	○	○
HSR-160^M	AB	AB	30 (15)	26 (30)	4 (15)	22.046	<0.001	●	●
HSR-176	AB	AA	25 (24)	23 (24)	---	0.021	0.885	○	●
HSR-177	AB	AB	3 (4)	10 (8)	3 (4)	0.586	0.746	○	○
HSR-179^M	AA	AB	53 (30)	7 (30)	---	33.759	<0.001	◎	○

suggests that neither plugs nor inserted paracymbial processes are associated with a reduction in female remating.

While the frequency of multiple paternity in *P. viridans* populations may be greater than that indicated by our limited data set, it is possible that the frequency may not be considerably greater due in part to changes in mate availability associated with a seasonal shift toward a female-biased sex ratio, as documented for a *P. viridans* population in Mérida, México (Arango et al. 2000). Thus, females reaching adulthood later in the year at this site presumably had access to fewer male suitors, a pattern also seen with the orb-weaving spider *Nephila clavipes* (Linnaeus 1767) due to a similar sex ratio shift (Higgins 2000). If a seasonal, female-biased sex ratio shift also occurs in southern California *P. viridans* populations, this may be one reason why even more broods did not exhibit evidence of multiple paternity in this study, particularly those whose mothers possessed neither plugs nor paracymbial processes (CC-15, HSR-148, HSR-177, Table 1).

The analysis of mating and parentage in *P. viridans* would greatly benefit from the future use of one or more hypervariable molecular markers applied to females, their spermathecal contents, and broods (e.g., Simmons et al. 2007) because such an approach would estimate both female remating rates and patterns of sperm utilization in natural populations. In addition, since copulatory plugs are often externally visible when they are present in the epigynum, if females were inspected for the presence of plugs on a daily basis (e.g., Kim & Choe 2007) prior to such genetic analyses, it might also improve our understanding of the frequency, persistence, and influence of this structure on opportunities for multiple mating by females.

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SHORT COMMUNICATION

A new approach to examining scorpion peg sensilla: the mineral oil flood technique

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Abstract. All scorpions possess jointed, ventral appendages called pectines. These organs have chemosensory, peg-shaped sensilla that detect substrate-borne chemicals. Previous physiological studies show that neurons within peg sensilla respond to an assortment of volatile organic chemical stimulants blown across the sensillar opening. We developed an improved method of chemical stimulant delivery called the mineral oil flood technique to further investigate the neural circuitry of scorpion pectines. The new mineral oil flood technique allows us to deliver chemical stimulants directly to individual sensilla by introducing a polar, liquid substance under non-polar mineral oil. Unlike previous methods of stimulant delivery, the mineral oil flood technique allows for precise control over the duration of direct contact between a liquid stimulant of known concentration and a sensillum.

Keywords: Pectines, chemosensory, electrophysiology, stimulant, Scorpiones

Pectines are movable sensory appendages that extend from the mid-ventral surface of all scorpions (Cloudsley-Thompson 1955). They are similar in function to the antennae of mandibulate arthropods that detect airborne chemicals (Kaissling 1987; Itagaki & Hildebrand 1990), except that pectines are ground-directed and respond to substrate-borne chemicals. When scorpions move in their environment, the pectines sweep intermittently against the substrate to detect food (Krapf 1986; Skutelsky 1995) and pheromones (Gaffin & Brownell 1992, 2001).

The primary sensory elements on pectines are hundreds of minute structures called peg sensilla, which adorn the ground-facing surface of each pectinal tooth (Carthy 1966, 1968; Ivanov & Balashov 1979; Foelix & Müller-Vorholt 1983). Each peg sensillum has a single slit-like pore that allows chemical stimulants access to receptor neurons inside the peg shaft. Approximately 10 sensory neurons innervate each peg (Foelix & Müller-Vorholt 1983), and synaptic contacts exist between neurons within a sensillum (Gaffin & Brownell 1997a).

Peg sensilla respond with different neural activity patterns to alcohols, aldehydes, ketones, and esters (Gaffin & Brownell 1997b). The sensilla were stimulated indirectly through puffs of stimuli blown across entire peg fields (Gaffin & Brownell 1997b) or by static clouds of volatile organic compounds brought near the peg tips (Gaffin & Walvoord 2004). Although these methods of stimulus delivery elicit neural responses, they have limitations. Most importantly, it is impossible to introduce a stimulant to a single peg sensillum without stimulating its neighbors. There is also no way, as such, to tell if stimulating a neighboring sensillum influences the response of the recorded sensillum. In addition, the concentration of stimulant reaching the sensillar pore is unknown, and removal of the stimulant from the peg field is uncontrollable.

To overcome these limitations, we developed an improved method of chemical stimulus delivery called the mineral oil flood technique, which uses non-polar mineral oil as a medium for delivering polar, liquid stimulants to an individual sensillum. In this study, we describe our new method and compare spontaneous and chemically-induced neural activity of peg sensilla in air and under oil.

Mature female *Paruroctonus utahensis* (Williams, 1968) (Scorpiones: Vaejovidae) collected from Crane County (31°28′59″N, 102°40′38″W), Texas, in March of 2008 were the animals used for this study. We individually housed each scorpion in 3.8 l glass jars containing approximately 250 ml of sand from the scorpion's collection site. The animals were on a 15:9 h L:D cycle and kept in a room with a steady temperature and relative humidity (22° C, RH 55–60%). We fed each scorpion one early instar cricket biweekly and watered the sand of individual containers with 5 ml of deionized water twice a week. At the conclusion of our study, we deposited a voucher specimen in the Sam Noble Oklahoma Museum of Natural History (OMNH–16279).

For preparing a scorpion for electrophysiological study, we briefly anesthetized a live animal by cooling it for two minutes inside a freezer at −5° C. Then we immobilized the animal with modeling clay on a microscope slide (7.62 × 2.54 cm), ventral side up to expose the pectines. We positioned a modified cover glass (5 × 18 mm) with walls of wax approximately 1 mm high caudal to where the pectines join the body (Fig. 1a). A notch in the wax wall allowed access of a pecten to the chamber (Fig. 1b). We secured the pecten spine and teeth to the cover glass with double-sided adhesive tape and fine application of less than 5 µl of quick-drying adhesive glue (Instant Krazy Glue™). Next, we applied additional wax to the area where the pecten spine crossed the edge of the cover glass to complete the wall of the chamber. We then placed one drop of mineral oil (≈5 µl) over the pecten with a 0.25 ml syringe (Fig. 1c). We secured the animal onto an adjustable platform and located peg fields with a high-powered compound microscope equipped with epi-illumination (Olympus BX50-WI). Lastly, we used an electrolytically-sharpened tungsten electrode to make extracellular recordings of chemosensitive neurons within individual sensilla (Gaffin & Brownell 1997b).

To record peg neurons, we digitized electrical activity with an analog to digital converter (1401-plus digitizing hardware, CED, Cambridge, England) and analyzed the record using Spike 2 laboratory software (CED, Cambridge, England). Impulses ("spikes") from each spontaneously active chemosensitive neuron were identified and separated into three classes (A1, A2, and B), based on the characteristic spike waveforms of the impulses from each neuron (Gaffin & Brownell 1997b). We used auto-correlation analysis to determine the purity of each spike class and cross-correlation analysis to detect synaptic interactions among spike classes (for details on auto- and cross-correlation analyses, see Gaffin & Brownell 1997a; Eggermont 1990).

To stimulate peg sensilla chemically, we filled a glass capillary tube (stimulant pipette) with an approximately 10 µm diameter tip with 95% ethanol (Gaffin & Walvoord 2004). We used a nonmetallic syringe needle (World Precision Instruments, Inc. MicroFil™ MF34G) to transfer the ethanol to the stimulant pipette. We then placed the stimulant pipette into a glass electrode holder positioned on a mechanical micromanipulator (Sutter Instrument Corp. 1140). For fluid stimulant introduction, we maneuvered the pipette tip with

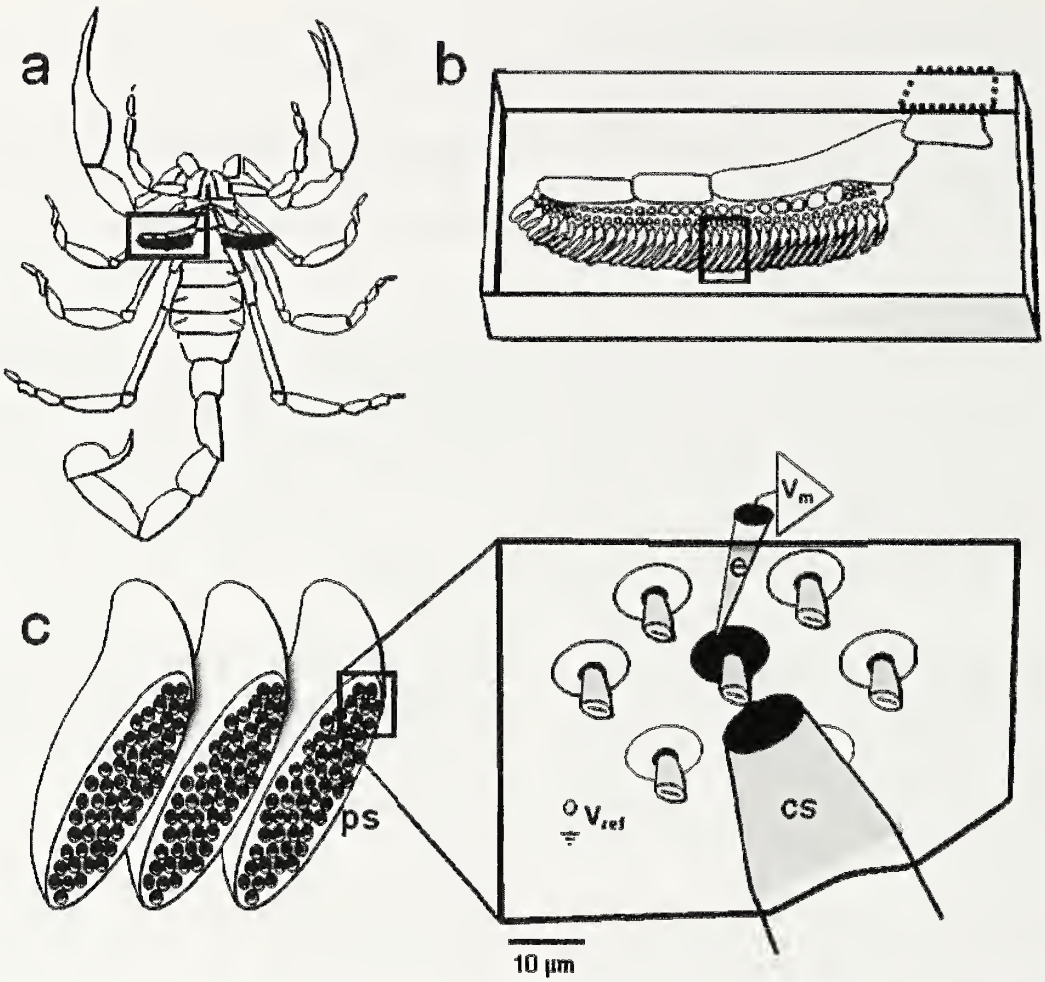

Figure 1.—Seorpion pecten configuration. a. The right pecten (outlined) as positioned for eleetrophysiological examination. b. Close-up view of the right pecten in a stimulation chamber. A piece of the wax barrier (dashed outline) is removed for pecten plaeement. c. Left, an expanded field of view of patches of peg sensilla (ps). Right, a mineral oil overlay of an expanded field of view of a peg field. A mieroelectrode (e) is inserted at the base of a single peg, under oil, to record baseline neural activity in the presenee or absence of a chemical stimulant (cs).

the micromanipulator so that it touched the pore of the recorded sensillum. Fig. 1c shows the general configuration of the peg field, microelectrode, and chemical stimulus delivery device during chemical stimulation.

In general, we were able to record spontaneous neural aetivity under oil for extended periods, some longer than six hours. Because the stability of a reeording often depended on the animal's inability to move its pecten, we improved the method of adhering the pecten to the cover glass. The most effective method was careful application of quick-drying adhesive to the pecten spine and the distal-lateral surface of each pectinal tooth. The least effective adhesives were paraffin wax and silicone gel.

Because the spread of mineral oil beyond the pecten and onto the animal's body induced pecten movement, we assembled a barrier between an isolated pecten and the rest of the animal. The most useful barriers were about one millimeter high, which still allowed easy access of an electrode and stimulant pipette to the sensillum, while preventing the spread of mineral oil beyond the pecten. The amount of mineral oil applied to the pecten also affected the quality of a preparation. Volumes of oil greater than or equal to 50 µl were not contained within the barrier. Excessive oil also blurred the field of view. In contrast, 5 µl of oil actually improved the resolution of the peg field; we could discern individual peg sensillar shafts, which is difficult when viewing sensilla in air. In addition, 5 µl of oil remained within the barrier and provided sufficient overlay for stimulant introduetion.

The presenee of mineral oil on peg sensilla did not affect baseline neural aetivity. Fig. 2 compares the spontaneous firing pattern of a peg sensillum in air with that of another peg sensillum under oil. Cross-correlation analyses reveal the same synaptie interactions in each record: when spike B fired, it inhibited spikes A1 and A2 for approximately 0.1 s.

Using the mineral oil flood technique, we confined ehemical stimulation to a single peg sensillum and controlled the onset and removal of the stimulant. For example, the right panel of Figure 3 shows the introduction and removal of liquid ethanol to a peg sensillum under oil for durations of one, two, and three seconds. Stimulations were conseeutive and spaced approximately 20 s apart, which produced reeeptor adaptation in the third response. In contrast, the left panel of Fig. 3 shows a prolonged neural recovery after introduction of a drop of ethanol to a peg sensillum in air. The time-expanded view of stimulant introduction shows the extent of record disturbance; reeorded electrical activity was inconsistent aeross all stimulations.

Our study represents the first account of selective ehemical stimulation of individual scorpion peg sensilla with a known concentration of aqueous stimulant. Because the presence of mineral oil over a sensillum did not affeet the baseline neural activity, we used

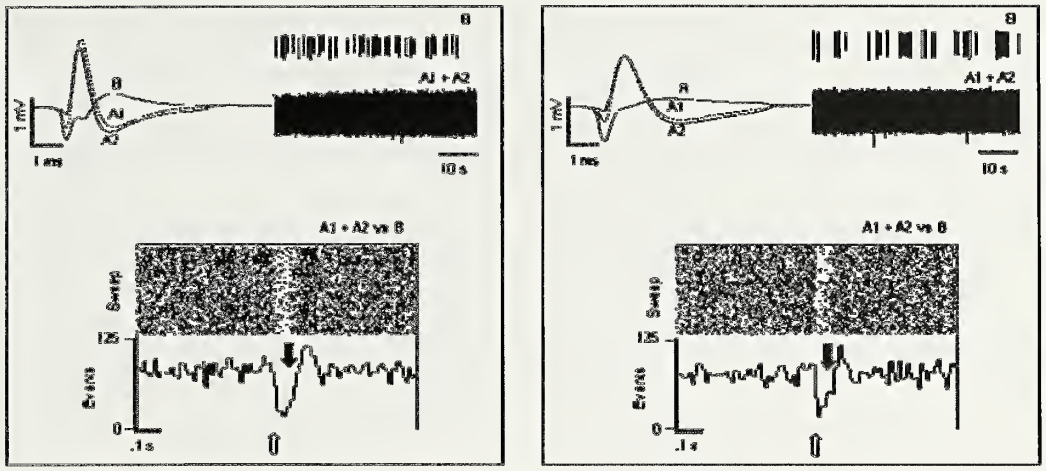

Figure 2.—Processed recordings of spontaneous neural activity. Shown are recordings of chemosensitive neurons (B, A1, and A2) from a sensillum in air (left panel) and a different sensillum under oil (right panel). Each line represents a neural impulse within the parsed 50 s record. The enlarged figures at the left of each recording indicate the B, A1, and A2 impulse waveforms. Cross-correlograms reveal that the firing of B (white arrow) inhibits the activity of A1 and A2 (black arrow) both in air and under oil.

oil as a medium through which to directly stimulate individual pegs with 95% ethanol for controlled durations.

One limitation of the mineral oil flood technique is that we can only use polar liquids as stimulants. Non-polar stimulants would mix with non-polar mineral oil. Therefore, future studies on peg sensillar function will use varying concentrations of polar solutions, such as salts and organic compounds that contain fewer than three carbons as stimulants.

In forthcoming experiments, we will use the mineral oil technique to further our understanding of peg sensillar function. This new method should generate the quantifiable data necessary for comparing sensillar neural responses. For example, we aim to stimulate many peg sensilla individually to compare response intensities to varying concentrations of stimulants. This will help us determine if all peg sensilla are functionally equivalent and if they follow dose-dependent response patterns. Additionally, no studies to date have tested for possible peg-to-peg interactions. We plan to test for synaptic interactions between neurons of neighboring sensilla by stimulating one peg sensillum while recording electrophysiologically from a neighboring sensillum. If synaptic interactions extend beyond neurons of an individual sensillum, we should observe a change in neural activity of the recorded sensillum as we stimulate its neighbor. Such a situation would provide evidence of lateral inhibition, which is a form of peripheral processing seen most commonly in the vertebrate retina.

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We thank Drs. Mariëlle Hoefnagels, Don Wilson, and Ari Berkowitz for their helpful suggestions and use of equipment. We also thank Nataliya Popokina for assistance in collecting and maintaining our animals. Finally, we thank the Life Fund of the University of Oklahoma Foundation and the OU Zoology Adams Scholarship Fund for providing support for this work.

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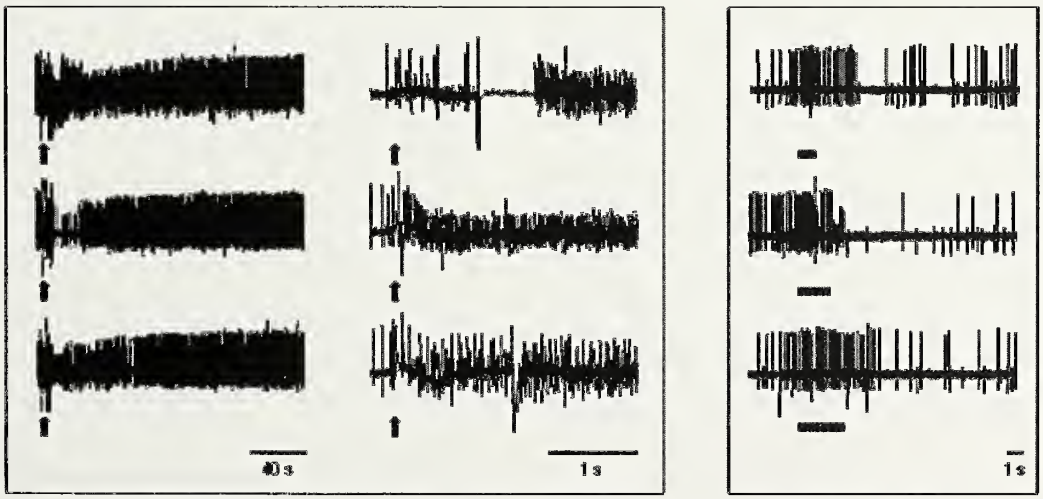

Figure 3.—Unprocessed recordings of neural activity during chemical stimulation. In air (left panel), fluid ethanol was introduced to a peg sensillum at three successive occasions (arrows). To the right of each record is a time-expanded view of the exact moment of stimulant contact with the recorded sensillum. Under oil (right panel), fluid ethanol was reversibly applied to a peg sensillum for 1, 2, or 3 s (horizontal bars).

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SHORT COMMUNICATION

New data on *Theridion italiense*, with description of the unknown female

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Abstract. I describe the female and redescribe the male of *Theridion italiense* Wunderlich 1995 from live and alcohol-preserved material and provide notes on the ecology, distribution, and affiliations with the very similar *Theridion uhligi* Martin 1974 and *Theridion petraeum* L. Koch, 1872.

Keywords: Araneae, Theridiidae, Carpathian Mountains

After recent taxonomists have transferred some species to other genera (Koçak & Kemal 2008, Wunderlich 2008), genus *Theridion* contains 47 species in Europe (Platnick, 2009). About a third of these species are poorly known, ten being described only from females and four known only from males. All of the poorly-known species have been discussed only in their original descriptions, so little is known about their distribution, habitat preferences, biology, and ethology. In some cases, even the original descriptions are wanting. This article sheds light on one such poorly known species.

Theridion italiense Wunderlich 1995 was described from a single male specimen collected in Abruzzo National Park in Italy (Wunderlich 1995), and other specimens have not been recorded since. This dearth of observations is probably due to its habitat preferences: low vegetation (5 to 10 cm above ground level) in rocky areas, which makes the webs difficult to see and the spiders nearly inaccessible to collecting with an insect net. However, I have collected 30 individuals from 25 m^2 of proper habitat in about 2 h, demonstrating that the species may not be as rare as it has originally appeared, given its near absence from the European arachnological literature.

The first specimens from Romania were gathered by hand and sweeping with an insect net in 2007. The spiders collected in 2008 were all captured by hand. All the material was preserved in 70% ethanol. Color of the specimens is described for both live and alcohol-preserved specimens. All measurements are in millimeters. The drawings were made with a drawing tube using an I.O.R. ML-4 microscope and an I.O.R. stereomicroscope.

The specimens examined in this study are deposited in the following collections: the National Museum of Natural History "Grigore Antipa" in Bucharest (MGAB), the Zoological collection of the Faculty of Chemistry-Biology-Geography in Timisoara (CBGT), the author's personal collection (CID), and the Thaler-Knoflach collection (CBK).

TAXONOMY

Family Theridiidae Sundevall 1833
Genus *Theridion* Walckenaer 1805
Theridion italiense Wunderlich 1995:691–695, figs. 8, 9
(Figs. 1, 2A, B, 3A, 4A, B, C, D)

Type material.—Holotype male: ITALY: Abruzzo National Park (1♂) collected in July 1994 (leg. Jörg Wunderlich) (in the collection of Jörg Wunderlich - CJW) – not examined.

Material examined.—ROMANIA: 1♀ Caras-Severin: Chiacotu Mic: Berzasca River Basin (44°43′52″N, 22°06′01″E) hand collecting, 1 May 2007, Ioan Duma leg. (MGAB); 1♀ Caras-Severin: Băile Herculane (44°52′00″N, 22°26′05″E) hand collecting, 10 May 2007, Ioan Duma leg. (MGAB); 4 ♀♀ Alba: Rîmetea (46°27′12″N, 23° 34′46″E) sweep net, 27 May 2007, Ioan Duma (CID); 25 ♀♀, 5 ♂♂ 14 May 2008, hand collecting, same location and collector (1♂, 3♀♀ in MGAB; 1♂, 1♀ in CBK; 1♂, 1♀ in IDC; 2♂♂, 20♀♀in CBGT).

Diagnosis.—Based upon the morphology of the palp and epigynum, *Theridion italiense* closely resembles *Theridion petraeum* L. Koch 1872 and *Theridion uhligi* Martin 1974. Both male and female *T. italiense* have dimensions similar to *T. uhligi*, but are clearly smaller than *T. petraeum*. Males' palps differ in the shape of the median apophysis (Fig. 3A–C). In females, the copulatory ducts are separate over their entire length. Using this characteristic feature, females of *T. italiense* can be easily differentiated from *T. uhligi*, whose copulatory ducts unite before opening in the center of the epigynum. The difference between female *T. italiense* and *T. petraeum* is that in the latter species the copulatory ducts open in the corners of the epigyne, not in its center.

The species also differ in their habitat preferences. *Theridion italiense* was found at low altitudes (350–1000 m) in limestone mountains. *Theridion petraeum* appears to be a species of higher altitudes and *T. uhligi* an inhabitant of grassy vegetation in the low plains.

Description.—*Male:* Dorsal carapace yellowish-brown with narrow black median band. On lateral sides of thoracic part, narrow black lines present as in *Theridion uhligi* Martin 1974, which largely disappear in some preserved specimens and thus may be hard to see. Sternum yellowish with grayish-black lateral margins. Labium brownish-red in contrast to *T. uhligi*, on which it is yellowish. Clypeus yellowish. Abdomen: dorsal part pinkish in live specimens, with median whitish-pink area bordered by a narrow brown line. In alcohol, pink disappears quickly and turns to whitish-yellow. Lateral parts of the abdomen pinkish with small white spots. Epigastric region large and brown, in contrast to that of *T. uhligi*, which is yellowish. Occasional long hairs present on abdomen. Legs: yellowish with brown annulations on femora, tibia, and tarsus. Annulations of leg segments may not be visible in some specimens. Leg lengths: leg I: femur 1.85–2, patella 0.5–0.55, tibia 1.7–1.8, metatarsus 1.65–1.8, tarsus 0.7–0.75, total leg length 6.4–6.9; leg II: femur 1.3–1.4, patella 0.5–0.55, tibia 0.85–0.92, metatarsus 1.0–1.12, tarsus 0.55–0.6, total leg length 4.2–4.6; leg III: femur 0.85–0.95, patella 0.35–0.4, tibia 0.5–0.55, metatarsus 0.7–0.75, tarsus 0.45–0.5, total leg length 2.85–3.15; leg IV: femur 1.3–1.4, patella 0.4–0.45, tibia 0.9–0.95, metatarsus 1.1–1.17, tarsus 0.5–0.57, total leg length 4.2–4.54. Tibial spines: 2:2:1:2. Chelicerae yellowish-brown without visible teeth. Basal hump on the frontal side of the chelicerae visible in lateral view (Fig. 2A). Palp (Fig. 1): Palp of *T. italiense* very similar to that of *T. uhligi* and *T. petraeum*, but rounder in ventral view and smaller than in closely related species. Shape of median apophysis (Fig. 2A) very different from that of *Theridion petraeum*, which has stiekle shape (Fig. 2C). Longer than that of *Theridion uhligi*, with differently shaped base. Also, on inner face of median apophysis in *T. italiense*, characteristic protuberance present visible from slightly prolateral angle as a

Figure 1.—Ventral view of the right palp of *Theridion italiense* male. Scale = 0.1 mm.

second, smaller apex (Fig 1). Somatic features (n = 5): total length 2.9–3.1 mm. Prosoma 1.0–1.15 mm long and 0.9–0.97 mm wide.

Female (Fig. 3C): Carapace yellowish with black median band extending to posterior row of eyes. Median band narrower in thoracic part of prosoma than in cephalic part, narrower in younger females and widening with age. This pattern also occurs on the lateral bands. Clypeus yellowish with a black triangular spot in front of chelicerae. Labium brownish. Chelicerae yellowish. Sternum yellowish with black bands on the sides. Width of bands varies with age of female (narrower in younger specimens and wider in older ones). Abdomen: dorsally whitish medially, bordered by narrow, sinuous pinkish-brown line. Lateral parts pinkish with small white spots. Pink color disappears in specimens preserved in alcohol and becomes whitish-cream. On ventral part of the opisthosoma, directly in front of spinnerets, triangular or square shape black mark bordered by short white lines on each side. Legs: yellowish with annulations on all segments except coxae. Annulations not as well defined in young females as in older ones. Leg lengths: leg I: femur 1.55–1.9, patella 0.4–0.5, tibia 1.2–1.5, metatarsus 1.0–1.3, tarsus 0.65–0.8, total leg length 4.8–6.0; leg II: femur 1.15–1.4, patella 0.3–0.4, tibia 0.65–0.8, metatarsus 0.9–1.1, tarsus 0.55–0.7, total leg length 3.55–4.4; leg III: femur 0.7–1.0, patella 0.25–0.35, tibia 0.45–0.6, metatarsus 0.55–0.7, tarsus 0.4–0.5, total leg length 2.35–3.15; leg IV: femur 1.2–1.5, patella 0.4–0.5, tibia 0.9–1.1, metatarsus 1.0–1.2, tarsus 0.45–0.6, total leg

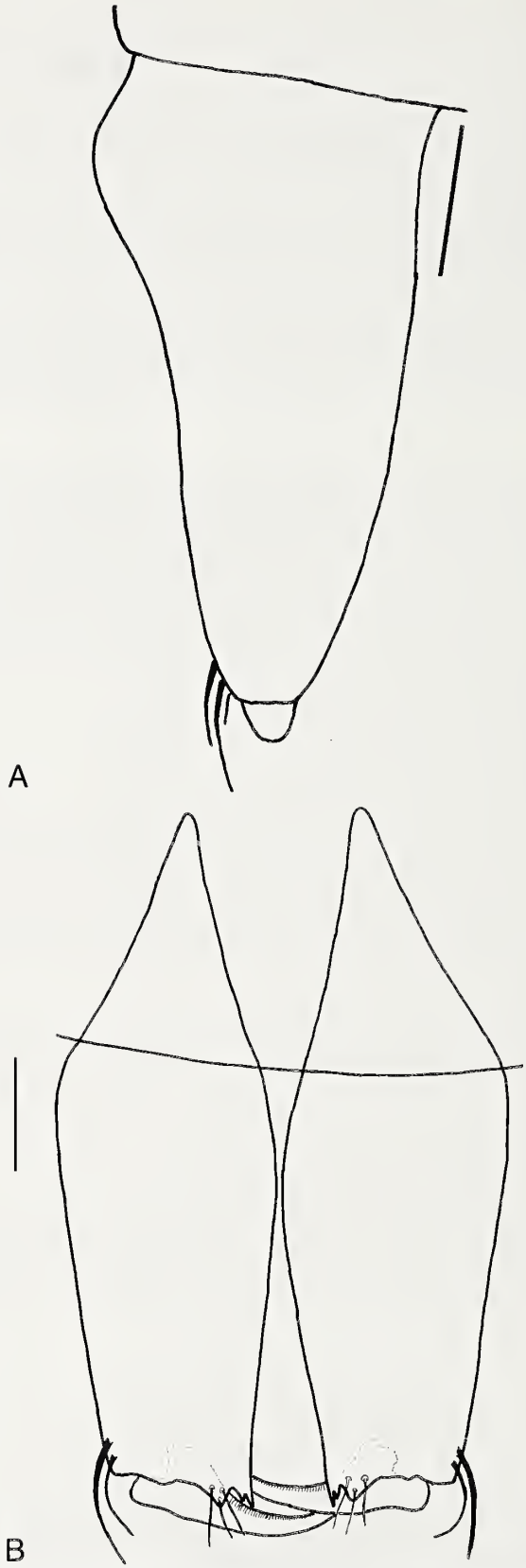


Figure 2.—Chelicerae of *Theridion italiense*. A. Lateral view of the male left chelicera; B. Frontal view of the female chelicerae. Scale = 0.1 mm.

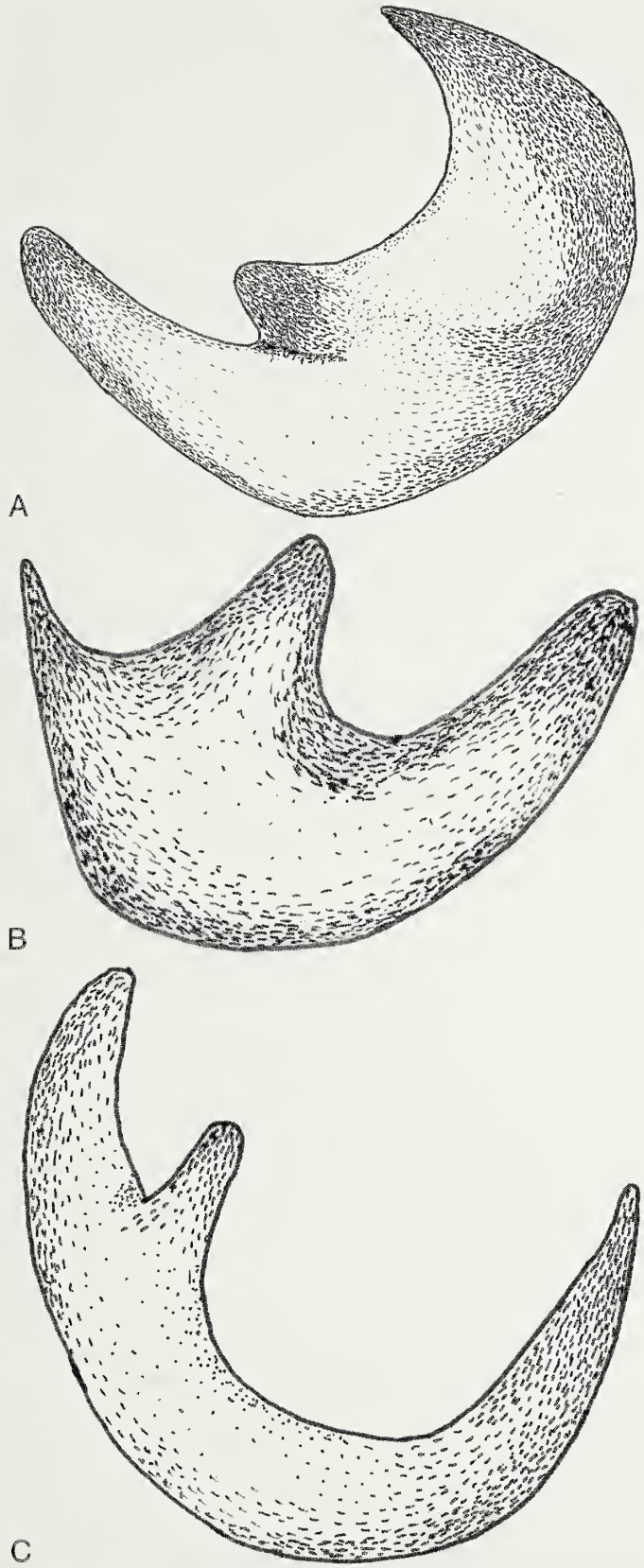


Figure 3.—Median apophyses. A. *Theridion italiense* (inner side, ectal view); B. *Theridion uhligi* (external side, mesal view); C. *Theridion petraeum* (external side, mesal view).

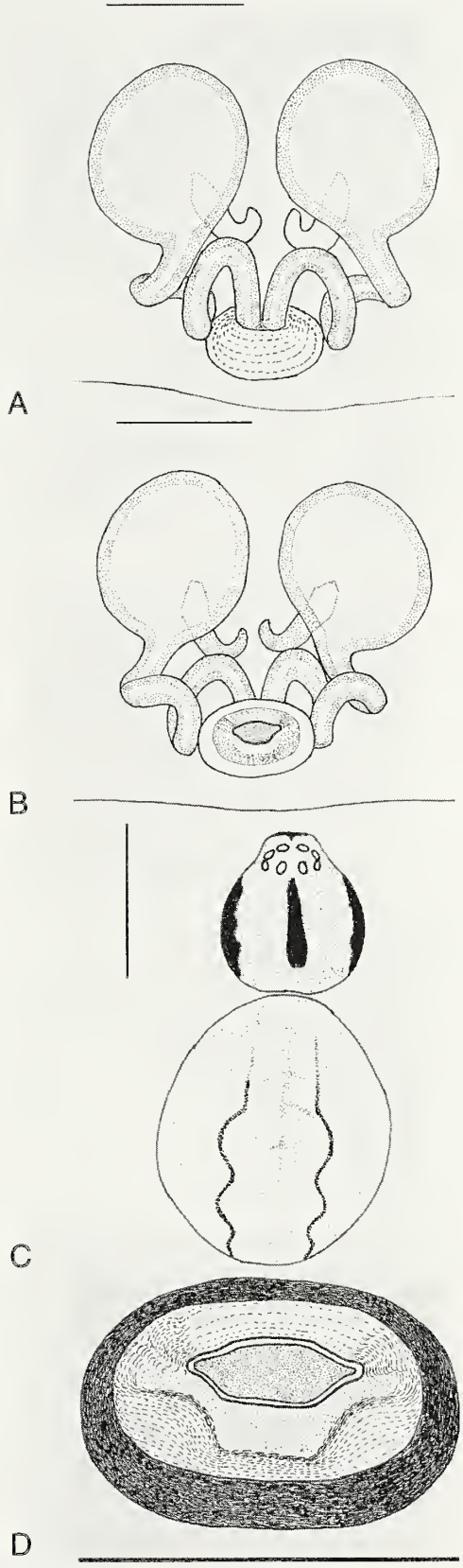
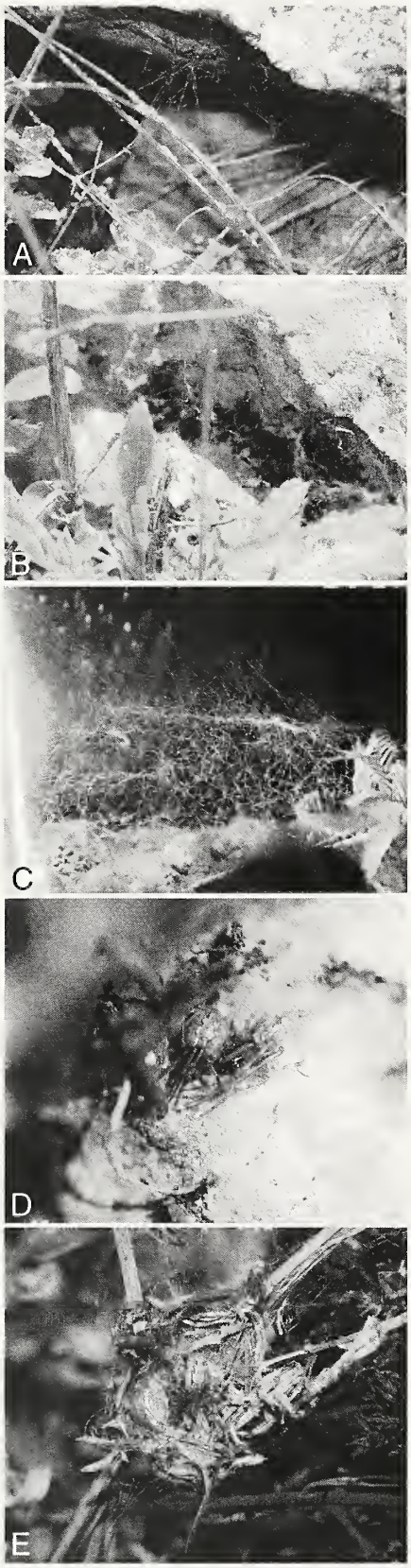


Figure 4.—Female *Theridion italiense* from Romania. A. Vulva (ventral view); B. Vulva (dorsal view); C. Habitus (dorsal view); D. Epigynum (Scale = 0.1 mm for A, B, D; 1 mm for C).



Figures 5.—Web of *Theridion italiense*. A. Frontal view of a 1-day-old web; B. Lateral view of a web; C. Frontal view of a 1-month-old web constructed in captivity in a plastic jar with a simple retreat; D. Ventral view of a simple retreat placed under a stone with the adult male and female; E. Ventral view of a retreat constructed under the branch and leaves of a small plant with the female prepared to lay its cocoon.

length 3.95–4.9. Tibial spines: 2:2:1:2. Chelicerae (Fig. 2B): two small teeth with common base on prolateral margin. Basal hump also present, but less prominent than in males. Epigynum: oval with sclerotized margins, especially the posterior one (Fig. 3D). Openings of copulatory ducts approximately in its center very close to each other. Copulatory ducts completely separate over entire length, not merging in common duct as in *T. uhligi*. Spermathecae round with thin walls. Somatic features (n = 31): total length 2.4–3.0 mm. Prosoma 0.9–1.2 mm long and 0.7–1.025 mm wide.

Ecological notes.—All specimens from Romania were gathered in May and were already mature. The majority of females were found with plugged epigyna, suggesting that first matings take place early in the spring. However, since the holotype male was collected in July in Italy, I infer that the species may reproduce throughout the warmer months of the year. The results of the collecting trip of 14 May 2008 suggests a female-biased adult sex ratio, in which case males mate with more than one female in their lifetimes. However the behavior of three adult males in the presence of unmated and mated females suggests that mated females do not mate with other males, similarly to other species of the *Theridion varians* group (Knoflach 1998). My preliminary observations indicate that males avoid the webs of females with plugged epigyna.

Theridion italiense appears to prefer rocky, sunny places with adequate moisture. It feeds on small insects; small ants were the most frequently observed prey.

Habitat notes.—Specimens of *Theridion italiense* from Romania were collected from low altitudes in limestone mountains (350–1000 m) at the edges of *Fagus sylvatica* forests in open, sunny areas. This type of habitat is classified as dry calcareous grassland and steppe (code 34) according to the CORINE land cover project, or as alpine and subalpine calcareous grasslands (code 6170) according to the NATURA 2000 project (Doniţă et al. 2005). In this habitat *Theridion italiense* can be found in crevices of rocky walls, in small bushes, or in grassy vegetation, always close to the ground (about 10 cm above it), with the threads of the web attached to stony walls on one side, to grass on the other side, and to the ground at its base.

The web of this species is constructed very close to the ground, being well camouflaged under the branches and leaves of various plants. It reaches a height of about 5–15 cm. The spider always weaves its web under the lower branches of small woody or herbaceous plants, close to the vertical surface of a rock. In the upper part of the web, the threads are fixed to the underside of the leaves and on nearby stones. The web is typical of the cobweb weavers, being three-dimensional, irregular, and formed by densely tangled sticky and non-sticky lines. A series of gumfoot threads are attached to the ground in order to catch small terrestrial insects such as ants, small coleopterans, and even collembolans. The web looks simple at the begining of construction (Fig. 5A, B), but becomes progressively more complex due to the new threads that spider adds over time (Fig. 5C).

Individuals occupy the upper part of the web close to the stony wall or under the leaves of various plants. Before the last molt females construct in the upper part of the web a more or less coneshaped retreat close to or under rocks, leaves or branches of small plants (Fig. 5D, E). The retreat is made of nonsticky threads, as in *Echinotheridion otlum* Levi 1963, *Theridion nigroannulatum* Keyserling 1884 or *Theridion evexum* Keyserling 1884 (Eberhard et al. 2008) When shelters are constructed under leaves they are not curled as in *Theridion nigroannulatum* (Eberhard et al. 2008). The retreat is camouflaged with moss, various vegetal debris and small grains of sand. The female will stay in this shelter with an adult male (Fig. 5D) until the last molt, after which copulation takes place. Before laying the cocoon the female will enlarge this retreat so that it will be big enough to protect her and her cocoon (Fig. 5E). When disturbed, *T. italiense* will retreat into the upper part of the shelter rather than dropping onto the ground. This kind of defence is similar to that observed in *Theridion evexum* (Eberhard et al. 2008).

Distribution.—Until now, *Theridion italiense* has been found only in central Italy and in the southwestern Carpathian Mountains (Romania). The Eastern Alps and the Dinaric and Rhodope Mountains also have many karst formations and the same type of habitats as those where the species was found (Doniţă & al. 2005) so I infer that the species may also occur in the mountains of the former Yugoslavia and Bulgaria as well.

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SHORT COMMUNICATION

Capture efficiency of an ant-eating spider, *Zodariellum asiaticum* (Araneae: Zodariidae), from Kazakhstan

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Abstract. *Zodariellum asiaticum* (Tyschchenko 1970) is an ant-eating spider from Central Asia. Using five syntopically occurring ant species, namely *Cataglyphis aenescens*, *Formica cunicularia* (both Formieinae), *Messor aralocaspius*, *Tetramorium caespitum* (both Myrmicinae), and *Tapinoma erraticum* (Dolichoderinae) in a laboratory study of prey-capture behavior, I evaluated capture frequency, attack latency, number of attacks, and paralysis latency. Although spiders captured all five ant species, capture efficiency varied when spiders were tested with the different ant species, being highest when the spiders were tested with *F. cunicularia*. I concluded that small juvenile *Z. asiaticum* probably adapt to feed primarily on species of small dolichoderine and myrmicine ants and that large juvenile and the adult *Z. asiaticum* adapt to feed primarily on large formicine ants.

Keywords: Stenophagy, specialization, myrmecophagy, prey-capture behavior

Spiders are well known for being euryphagous predators, (i.e., consuming a wide variety of prey), but many have an aversion to ants. This makes any group of spiders that routinely eat ants ("myrmecophagy") especially interesting. Myrmecophagie spiders are of further significance because they often seem to adopt ant-specific prey-capture behavior, and they may actively choose ants in preference to other prey (e.g., Huseynov et al. 2008). There is particular interest in spider species that might be exclusively myrmecophagic.

Although myrmecophagy may be a rare phenomenon in spiders as a whole, examples are found in an assortment of spider families, including the Gnaphosidae, Oecobiidae, Salticidae, Theridiidae, Thomisidae, and Zodariidae (Glatz 1967; Heller 1976; Porter & Eastmond 1982; Jocqué 1991; Castanho & Oliveira 1997; Jackson et al. 1998). Species that appear to be exclusively myrmecophagic include a theridiid *Dipoena* and a thomisid *Aphantochilus* (Umeda et al. 1996; Castanho & Oliveira 1997). However, regardless of whether a species is exclusively or partially myrmecophagic, arachnologists have tended to envision myrmecophagic spiders as preying on ants in general rather than as having become adapted to particular kinds of ants. Yet there is evidence that at least some of the myrmecophagic spiders show a preference for certain genera or even species of ants within the family Formicidae as a whole. Researchers need to gather more information about myrmecophagic spiders so that we can determine how important the targeting of particular ant taxa is for these predators. "Targeting" includes a variety of adaptations by which a spider might specialize on particular kinds of ants, including adaptation related to morphology, physiology, and behavior.

The myrmecophagic spiders I investigated are from the family Zodariidae, one of the most diversified families of spiders (Platnick 2009). The family Zodariidae is known for including a number of myrmecophagic species (Jocqué 1991), but the natural history of the great majority of these species is still poorly known. Available evidence suggests the species in four of the genera in the subfamily Zodariinae, namely *Diores*, *Trygetus*, *Zodariellum*, and *Zodarion*, are exclusively myrmecophagous (Marikovsky & Tysehchenko 1970; Pekár et al. 2005; Haddad & Dippenaar-Schoeman 2006). However, evidence that the predator distinguishes prey below the level of family (Formicidae) has come from only one of these genera, namely *Zodarion* (Pekár 2005; Pekár et al. 2008).

Here I consider a species from the genus *Zodariellum*. The species in this genus are morphologically uniform (Marusik & Koponen 2001), meaning that distinctive interspecific differences are evident only in the details of sexual organ shape, not in structures such as the spider's chelicerae that function directly in predation. This suggests that, for finding evidence of adaptation to speeific types of ants, we should investigate behavioral and predatory-related physiologieal traits.

In this study, I focus on some traits related primarily to behavior. Ant-eating spiders typically capture ants using a 'bite-and-release' taetic (e.g., Jackson & van Olphen 1992; Cushing & Santangelo 2002). One probable advantage of this mode of attack is that it enables the predator to avoid being injured or killed when ants counter-attack. Using this mode of attack, species from the genus *Zodarion* can subdue a number of different kinds of ants, but apparently the spider's efficiency in capturing different kinds of ants varies considerably. Evidence that efficiency varies comes from data on paralysis latency (i.e., the time elapsing between when the ant is attacked and when it becomes immobile) and the frequency of attacks before the prey is eaten (e.g., Pekár 2005). These are the parts of the predatory sequence to which I paid particular attention in this study of *Zodariellum*.

Worldwide, there are 22 species in the genus *Zodariellum*, about 10 of which appear to be endemic to Central Asia (Platnick 2009). There are published anecdotal prey records for two species, *Z. asiaticum* Tyschchenko 1970 and *Z. sahariense* Denis 1959, feeding on ants (Pierre 1959; Marikovsky & Tyschchenko 1970). Marikovsky reported that the ant on whieh *Z. asiaticum* preys is primarily *Formica cunicularia* Latreille, but predation was also observed on *Tetramorium caespitum* (Linnaeus), *Messor aralocaspius* Ruzsky, and *Cataglyphis aenescens* (Nylander) (Marikovsky & Tyschchenko 1970; Marikovsky 1979).

Zodariellum asiaticum, the species I investigated, occurs in southeastern Kazakhstan. The specimens I used (eight female and seven subadult individuals; body lengths 3.5–4.5 mm) were collected in April on the sandy slopes of a semi-desert habitat along the Illi River, near the eity of Kapchagay (43°56′93.4N, 77°03′56.7E). Spiders were identified using Marikovsky & Tysehchenko (1970) and Marusik & Koponen (2001) and kept in glass tubes (diameter 10 mm, length 60 mm) with moistened substrate (plaster of Paris). All spider specimens are deposited in the collection of arachnids of the Department of Botany and Zoology, Masaryk University, Brno.

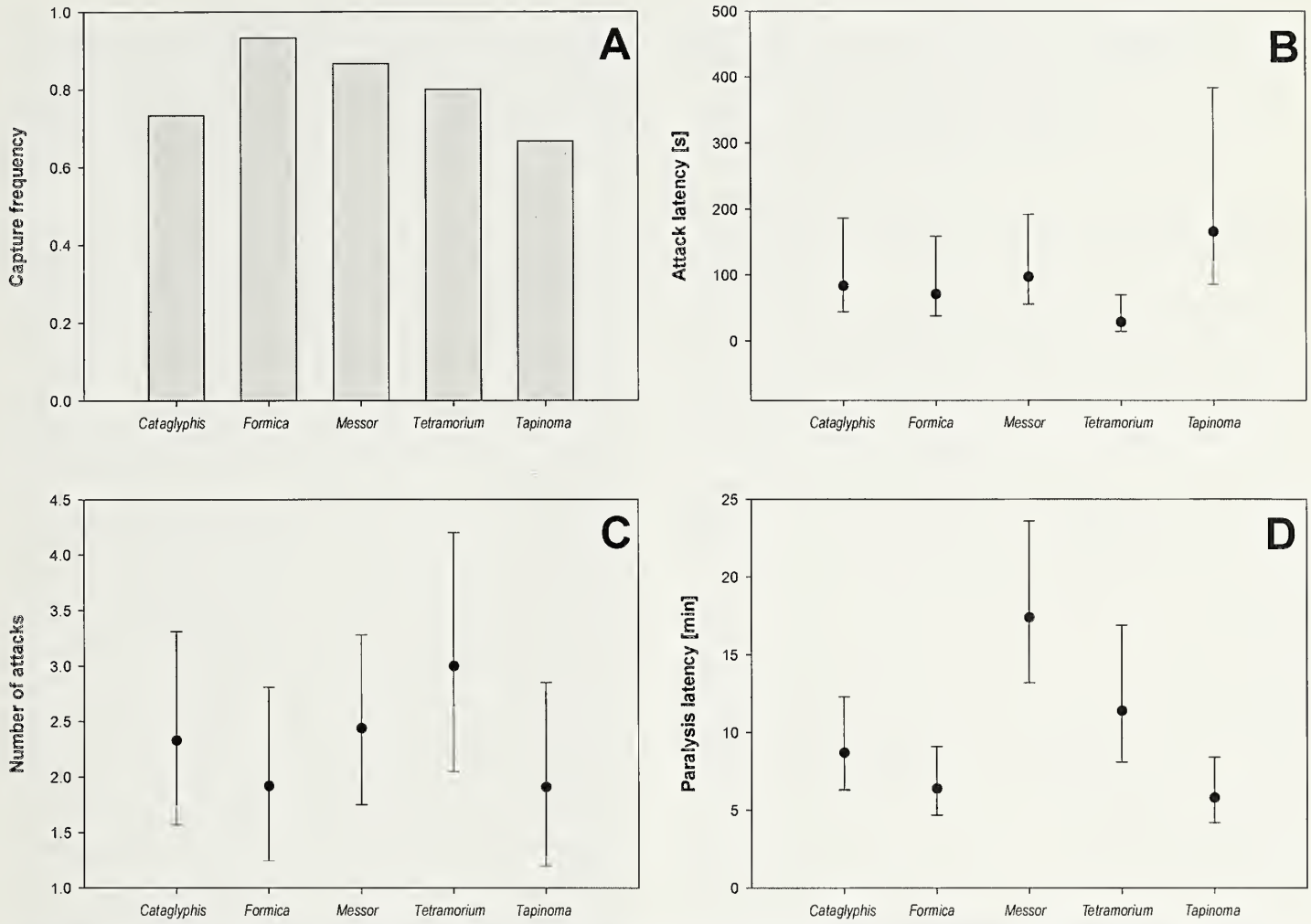

Figure 1.—Comparison of four predatory traits of *Z. asiaticum* for five ant species (from three ant subfamilies). A. Mean capture frequency. B. Mean latency to the first attack. C. Mean number of attacks. D. Mean latency to complete paralysis. Whiskers are 95% confidence intervals for means.

The ant fauna occurring syntopically with *Z. asiaticum* was surveyed at the same sites (ants identified using Marikovsky 1979). Five ant species were used in the experiments: *Cataglyphis aenescens* (body length 4.5–8 mm) and *Formica cunicularia* (4–6 mm) (both Formicinae), *Messor aralocaspius* (4.5–8 mm) and *Tetramorium caespitum* (3–3.5 mm) (both Myrmicinae), and *Tapinoma erraticum* (Latreille) (3.5–4 mm) (Dolichoderinae). We collected ants used as prey in the field a few hours before we used them in the experiment.

I chose one of the ant species (*Messor*) to serve as the standard for initial feeding (i.e., after being collected, the spiders were fed with a single individual of *Messor* the next day). Three days later, each spider was offered successively, in random order, a single ant from each of the five species. I tested each spider with each ant species only once.

There was a 2-day interval between successive trials. A single trial consisted of releasing an ant into a dish occupied by a spider (diameter 40 mm; filter paper glued to the bottom; thin layer of fluon on the sides). Each spider had been in the Petri dish for one day before the trial began. Spiders usually attacked within 30 s. If the spider did not attack the ant within 10 min, I terminated the trial. These aborted trials were classified as rejection of prey. For each trial, I recorded attack latency (i.e., the time between when the spider oriented itself toward the ant and the first attack), number of successive attacks, and paralysis latency (i.e., the time between the first attack and the prey becoming completely immobilized).

Data were analyzed using Linear Mixed-Effects Models (LME) from the NLME-package within the R-environment (R Development Core Team 2007). I chose this method because observations were not independent (i.e., there was repeated use of the spider individuals) and LME is designed for taking into account repeated measurements (Pinheiro & Bates 2000). Both latencies appeared to come from asymmetrical (skewed to the right) distributions. Therefore, I applied logarithmic transformation, after which the data distribution approximated the normal distribution. I expected that the size of ant prey might affect the number of attacks and the paralysis latency. Therefore, I used prey size as a covariate when analyzing the data. Frequency of capture was compared using the Cochran Q test.

Predatory sequences in encounters between *Z. asiaticum* and ants were similar to predatory sequences in encounters between species of *Zodarion* and ants (e.g., Pekár 2004). *Z. asiaticum* approached ants quickly from behind and attacked, usually by contacting the ant's dorsal thorax or leg, releasing the ant, and then continuing to attack several more times or waiting until the ant became paralyzed.

Spiders attacked all five ant species, the most frequently attacked species being *F. cunicularia* and the least frequently attacked being *T. erraticum* (Fig. 1A). However, differences in frequency of attacking ant species were not statistically significant (Cochran Q test, $Q_4 = 4.8$, $P = 0.31$). Differences in latency of making the first attack were also non-significant across ant species (LME, $F_{4,42} = 1.9$, $P = 0.13$, Fig. 1B) and for number of attacks (LME, $F_{4,42} = 0.7$, $P = 0.6$, Fig. 1C). When number of attacks were considered in relation to ant size, differences in the data were not significantly different (i.e., number of attacks is independent of ant size) (LME, $F_{1,42} = 0.07$, $P = 0.79$).

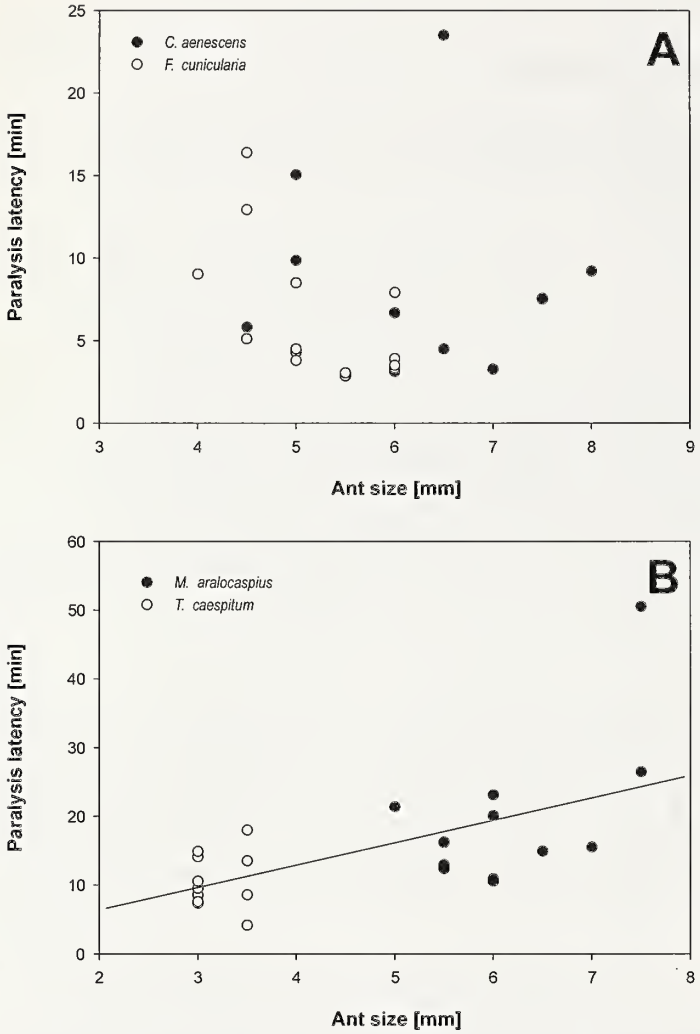

Figure 2.—Relationship between paralysis latency and the size of ants for two formicine (A) and two myrmicine (B) ant species. Linear model ($y = -0.78 + 3.33\ x$) is shown for myrmicine.

However, there were significant differences between ant species for paralysis latency (LME, $F_{4,42} = 10.8$, $P < 0.0001$, Fig. 1D), latency for *Messor* being about three times as long as latencies for *Cataglyphis*, *Formica* and *Tapinoma*. Latency for *Tetramorium* was similar to latency for *Messor* (Fig. 1D). For formicine ants (*Cataglyphis* and *Formica*), there were no significant differences in paralysis latency when considered whether latency depended on ant size (LME, $F_{1,23} = 0.14$, $P = 0.72$, Fig. 2A). For myrmicine ants (*Messor* and *Tetramorium*), on the other hand, latency for large ants was significantly longer than for small ants (LME, $F_{1,24} = 13.4$, $P = 0.001$, Fig. 2B).

I found that *Z. asiaticum* captured each of the five ant species used in this study, suggesting that this spider may have some general adaptations that enable it to be effective at capturing ants in general. Yet there are some critical differences indicating that this predator distinguishes ants below the level of family and has acquired adaptations by which it can be particularly effective as a predator of certain kinds of ants. Preference is a motivational trait that drives a predator's prey-choice behavior (Huseynov et al. 2008), and it seems likely that my data on capture frequency and attack latency are especially closely linked to the preferences of *Z. asiaticum*. I might predict that a predator will be more likely to attack and quicker to attack prey it prefers. However, my hypothesis is that paralysis latency and the numbers of attacks made by *Z. asiaticum* on different ant species are determined to a large extent by biochemical specificity of the spider's venom. If these predictions and hypotheses are correct, then I have no evidence from this study of preference for particular kinds of ants, nor does the number of attacks made before the ant is immobilized provide evidence of venom specificity. However, significant differences among ants were found for paralysis latency. The data for paralysis latency do not simply corroborate the venom-specificity hypothesis, but they add strength to this hypothesis and imply that further investigation would be of interest.

More specifically, paralysis data support a hypothesis that *Z. asiaticum* has adapted in special ways as a predator that targets formicine ants and *F. cunicularia* in particular. Stated more precisely, *Z. asiaticum* specializes on the formicine ants. In this context, "specialized" refers to having special characteristics (in this instance, formicine-specific venom) that make a predator especially effective at preying on a particular kind of prey.

It is of interest that no size-dependent relationship was evident for formicine ants. This is what we might expect if the venom of *Z. asiaticum* is specific to these ants. In an earlier study (Pekár et al. 2008), we demonstrated similar results for *Zodarion germanicum* (C.L. Koch 1837), as this species performed best, in terms of growth, development and survival, on a diet consisting of only formicine ants. For this species as for *Z. asiaticum*, there was no significant variation in paralysis efficiency dependent on ant size when the ants were formicines. It is also noteworthy that Marikovsky reported a paralysis latency of about 4 min when *Z. asiaticum* attacked *F. cunicularia* in the field (Marikovsky & Tyschchenko 1970), as this corresponds closely to the latencies I found in the laboratory.

Without further experiments, the connection between paralysis latency and venom specificity remains only a hypothesis. Paralysis latency may be influenced by variables that could not be controlled in this study. For example, the volume of venom injected may influence paralysis latency, but venom volume was not determined in this study. That venom volume needs to be considered is illustrated by recent findings from a study on an unrelated spider of the genus *Cupiennius*. This spider was shown to inject larger volumes of venom when it detected that the prey was especially dangerous (Wigger et al. 2002).

For the genus *Zodarion*, two distinct groups appear to be identifiable in the context of prey specificity. First, there are species that may normally exploit a single ant species. More specifically, there appear to be species that exploit solely ants from the genus *Messor*. Being polymorphic, ants of this genus may be feasible prey for exploitation by *Zodarion* throughout the spider's life cycle, as there would always be available, regardless of the developmental stage to which an individual of *Zodarion* might belong, a suitable size morph of this ant species (Pekár, unpublished). The other group consists of *Zodarion* species whose adults tend to prey most often on monomorphic ant species. I hypothesize that these *Zodarion* species, specifically the smaller juveniles, prey primarily on ant species that are smaller than the species on which the adults prey. This implies that the spiders adapt to the ants they target as they progress through their life cycles. Often the stage-specific switch may be from smaller to larger ant species belonging to the same ant subfamily (Pekár et al. 2008).

Field data suggest that *Z. asiaticum* frequently exploits ant species from different subfamilies. For *Z. asiaticum*, there may be a developmental adaptation in the ants primarily targeted, with small juveniles feeding on small dolichoderine ants such as *Tapinoma* and on small myrmicine ants such as *Tetramorium*, and with large juveniles and adults feeding primarily on large formicines such as *F. cunicularia*. On one occasion, I found a juvenile of *Z. asiaticum* feeding on *Tapinoma* in the field, which helps to support this hypothesis. If this hypothesis is corroborated, then it will be of interest to investigate the venom specificity of the early juvenile stages of *Z. asiaticum* to determine whether small juveniles have venom that is specific not to formicines, but instead to dolichoderines or myrmicines or both.

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